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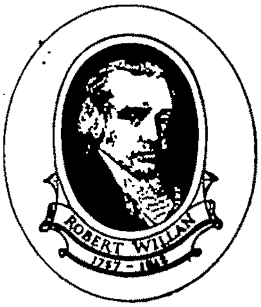

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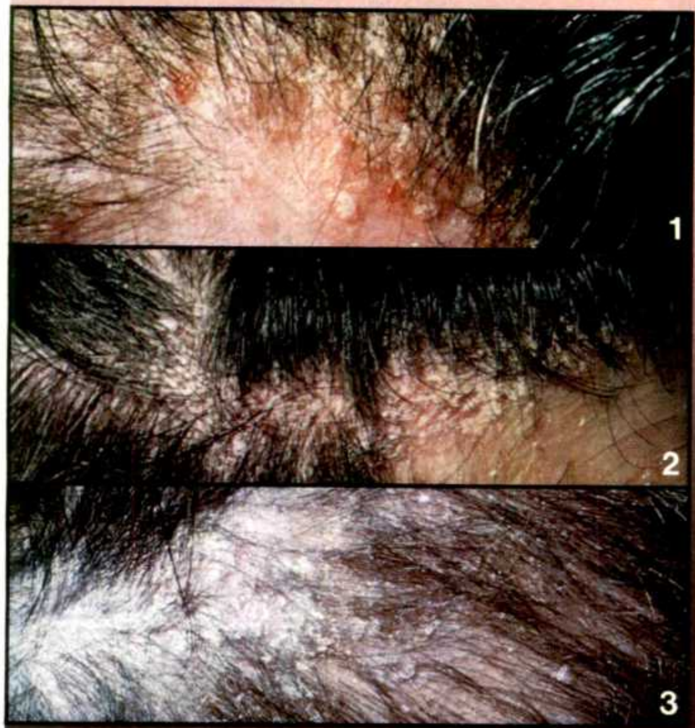

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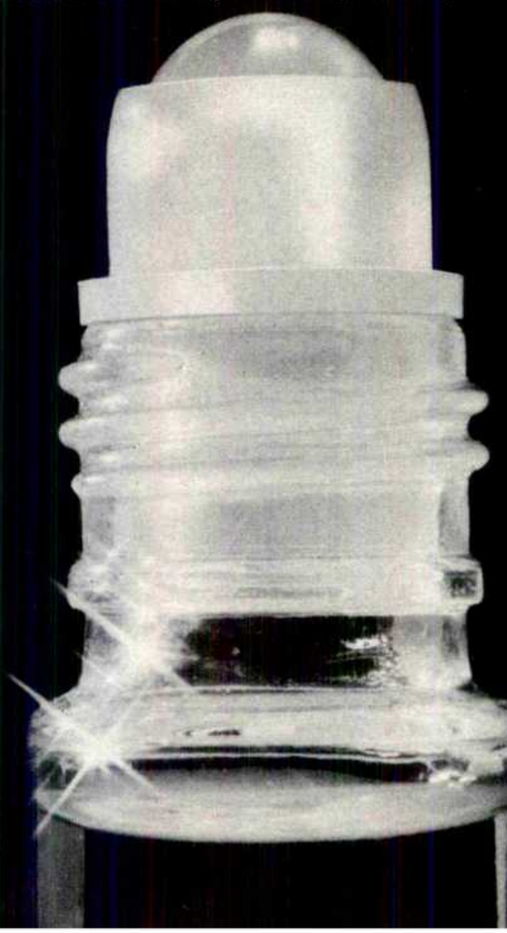

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For the topical treatment of hyperhidrosis specifically involving the axillae, hands or feet.

DIRECTIONS FOR USE:
Apply to the affected sites at night as required and allow to dry. Wash off in the morning.

PRECAUTIONS:
Restrict application to affected sites only. Keep away from the eyes.

PACKAGE QUANTITY AND BASIC NHS PRICE:
10ml glass roll-on bottle £2.15.

REFERENCE:
British Medical Journal, 1978, **2**, 84.

PL 0173/0030

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Why mess about with topical antifungals

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For fungal infections, topical therapy is often both messy and inconvenient – and is unlikely to be as completely effective as oral Grisovin.

Grisovin is carried in the bloodstream to the basal layer and gets right to the root of ringworm infections. It protects the newly formed keratin cells from infection and as they move outwards, the fungus is expelled.

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Why mess about with topical antifungals when Grisovin gets right to the root of the problem?

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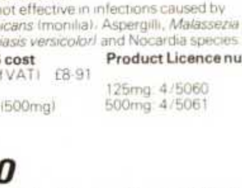

THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.

Children: usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

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Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost
(exclusive of VAT) £8·91
Bottle of
100 tablets (500mg)

Product Licence numbers
125mg: 4/5060
500mg: 4/5061

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Trent Regional Health Authority,
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Sheffield S10 5TR.

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(**clobetasol propionate**)

Even long-standing, stubborn eczematous lesions respond quickly to Dermovate – with rapid relief from itching; and this is often followed by prolonged periods of remission. And because Dermovate is so rapidly effective, treatment need be of short duration only, minimising the risk of steroid side effects.

Prescribing information

Dosage and administration
Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

Side effects
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism.

This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications
Rosacea, acne and peri-oral dermatitis, all of which can be treated with oral tetracyclines. Viral diseases of the skin, e.g., herpes simplex.

Basic NHS cost (exclusive of VAT)
Dermovate Cream or Ointment 25 gram tubes £1·74 (also availabl in 100 gram tubes).

Presentation	**Product Licence numb**
Dermovate Cream	4/0219
Ointment	4/0220

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And Alphosyl HC is preferable on safety grounds since it does not contain a fluorinated steroid.

Alphosyl HC

hydrocortisone 0.5% with allantoin and coal tar extract.

purpose-designed for psoriasis

*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol 33 No. 4, 106.

ntation A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone, 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogenous and is light tan in colour. Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage dministration** Apply two to four times daily massaging vigorously into the affected areas. **ra-indications and Warnings, etc.** In infants long-term continuous therapy should be avoided. Adrenal can occur even without occlusion. Topical administration of corticosteroids to pregnant animals can cause abnormality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. **Package Quantities** Tubes of 30gm and 45gm. **Basic NHS cost** £1.78 for 30gm, £2.52 for 45gm. **Product Licence No.** 0036/0026. Further information is available from

Stafford-Miller Limited, Professional Relations Division, Hatfield, Herts. AL10 0NZ

Face facts....

in acne

LOW-DOSE

Minocin

minocycline hydrochloride

50mg b.d.

is a significant improvement over ordinary tetracyclines

HIGH PENETRATION[1,2] EVEN INTO SEBUM.

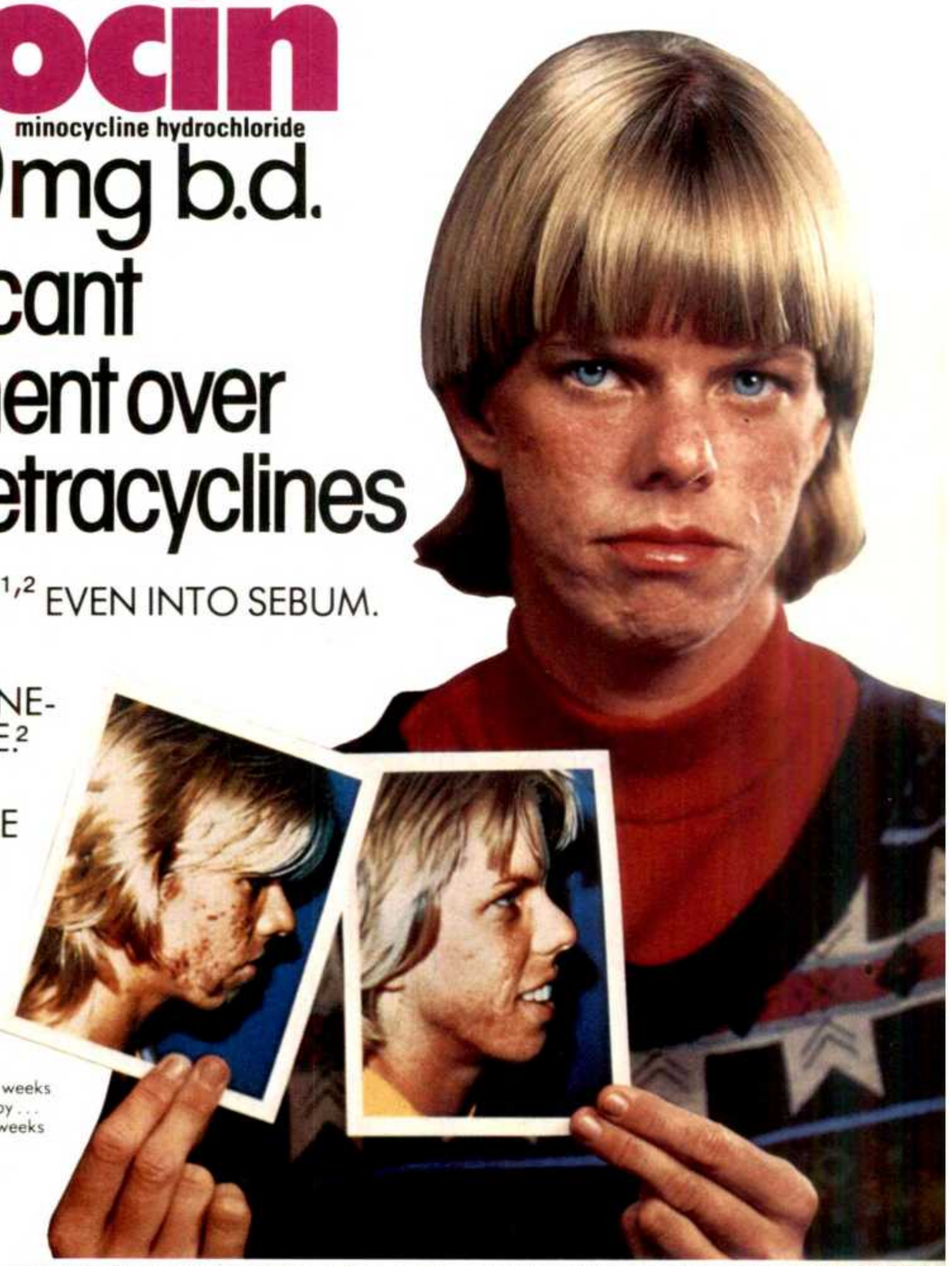
HIGH ACTIVITY
EVEN IN TETRACYCLINE-RECALCITRANT ACNE.[2]

SIMPLE DOSAGE
B.D. DOSAGE CAN BE TAKEN WITH MEALS.

LOW INCIDENCE OF SIDE EFFECTS[3]

1. Cullen, S.I., Cutis, 1978, **21**, 101
2. Becker, F.T., Cutis, 1974, **14**, (4), 610
3. Cullen, S.I. and Cohan, R.H., Cutis, 1976, **17**, 1208

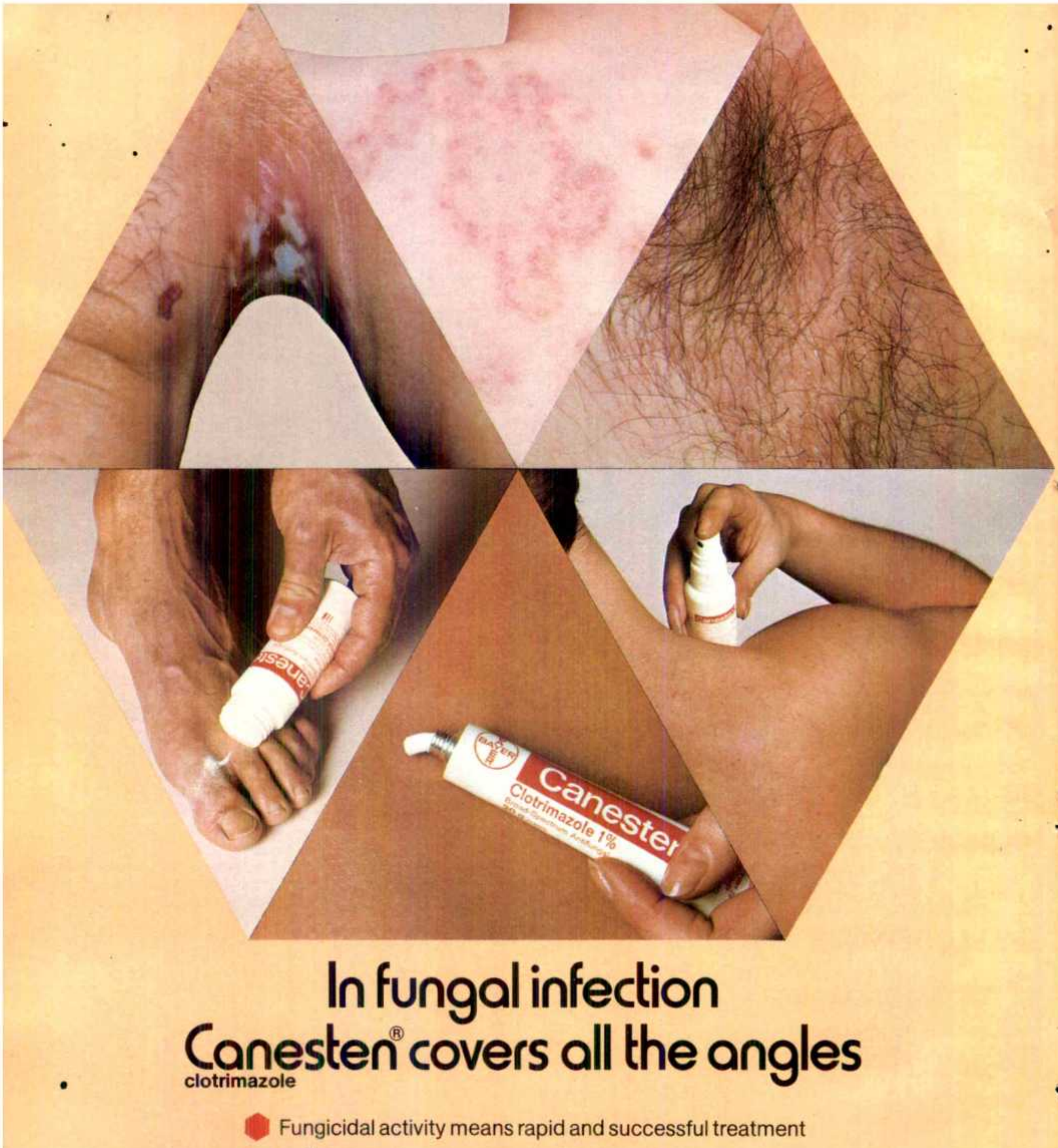
NEW LOW DOSE



Grade IV acne after 6 weeks of tetracycline therapy . . . and same patient 6 weeks after therapy was changed to Minocin.

Dosage Adults and children over 12 years. One tablet twice daily. Minocin may be taken with meals. Treatment of acne should be continued for a minimum of six weeks. **Contra-indication** Known tetracycline hypersensitivity. Systemic lupus erythematosus. **Precautions** Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. **Warnin and side effects** Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discolouration of teeth is a possible sequela of treatment during pregnancy or early childhood (up to 8 years of age). **Presentation** Beige tablets containing 50mg of minocycline as the hydrochloride, supplied in bottles of 100 at a basic NHS cost of £18.00. PL/0095/006 Full prescribing information is available on request. MINOCIN is a trademark.

Lederle Laboratories Division of Cyanamid of Great Britain Ltd, Fareham Road, Gosport, Hants PO13 0AS (Tel: 0329-236131).



In fungal infection
Canesten® covers all the angles

clotrimazole

- Fungicidal activity means rapid and successful treatment
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- Cost-effective routine antifungal therapy

escribing information: Presentations** *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white powder
ntaining 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections. **Dosage and Administration** *Cream,*
omiser Spray Apply to the affected area 2 - 3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic
ainst re-infection. **Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near a
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omiser Spray: PL 0010/0060 Powder: PL 0010/0067 **Basic NHS Prices** Cream: 20g, £1.52 50g, £3.55 Atomiser Spray: 40ml, £4.08 Powder: 30g, £1.10. Further information
available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. Distributed in the Republic of Ireland by
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(clobetasone butyrate)

a unique balance of efficacy and safety

Prescribing information

Uses

Eumovate is suitable for treating the
forms of eczema, seborrhoeic dermat
and other steroid responsive skin
conditions.

Dosage and administration

Apply up to four times a day until
improvement occurs, when the frequ
of application may be reduced.

Contra-indications

Bacterial, fungal or viral diseases of th
skin.

Precautions

In infants and children, long-term
continuous topical corticosteroid ther
should be avoided where possible, as
adrenal suppression can occur. In infa
the napkin may act as an occlusive
dressing, and increase absorption. Th
least potent corticosteroid which will
control the disease should be selected
Appropriate antimicrobial therapy sho
be used concurrently whenever treatir
inflammatory lesions which have becc
infected.
Any spread of infection requires
withdrawal of topical corticosteroid
therapy and, possibly, commenceme
systemic chemotherapy.
As with all corticosteroids, prolonged
application to the face is undesirable.
Topical steroids should not be used
extensively in pregnancy, i.e., in large
amounts or for prolonged periods.

Side effects

In the unlikely event of signs of
hypersensitivity appearing, application
should stop immediately. If large areas
were to be treated, some patients cou
absorb sufficient steroid to cause tran
adrenal depression despite the low de
of systemic activity associated with
clobetasone butyrate.
Local atrophic changes could occur w
moisture increases absorption of stero
but only after prolonged use.

Basic NHS cost (exclusive of VAT)
25 gram tube £1·48 (also available in
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Product Licence numbers
Cream: 4/0233
Ointment: 4/0254

Glaxo

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Further information on Eumovate (trad
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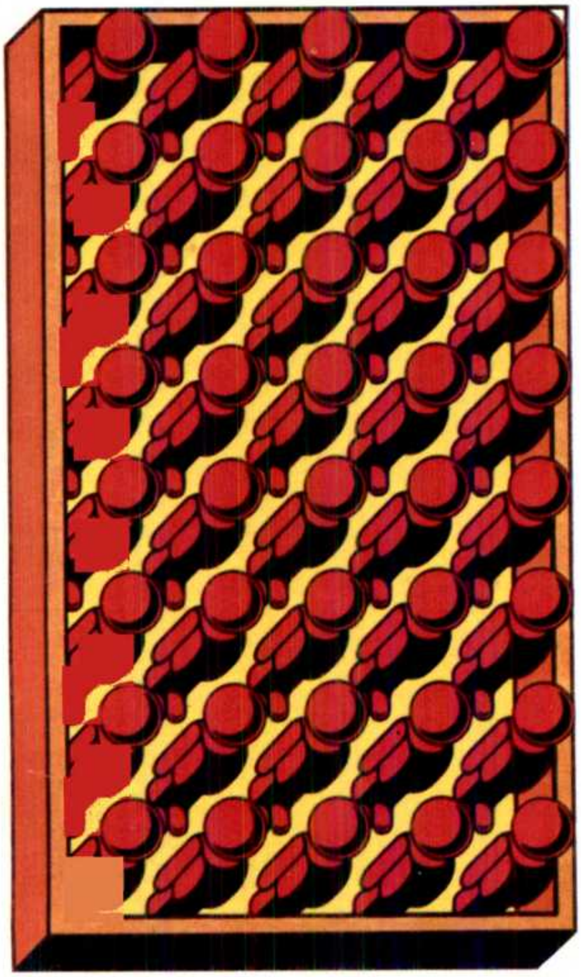
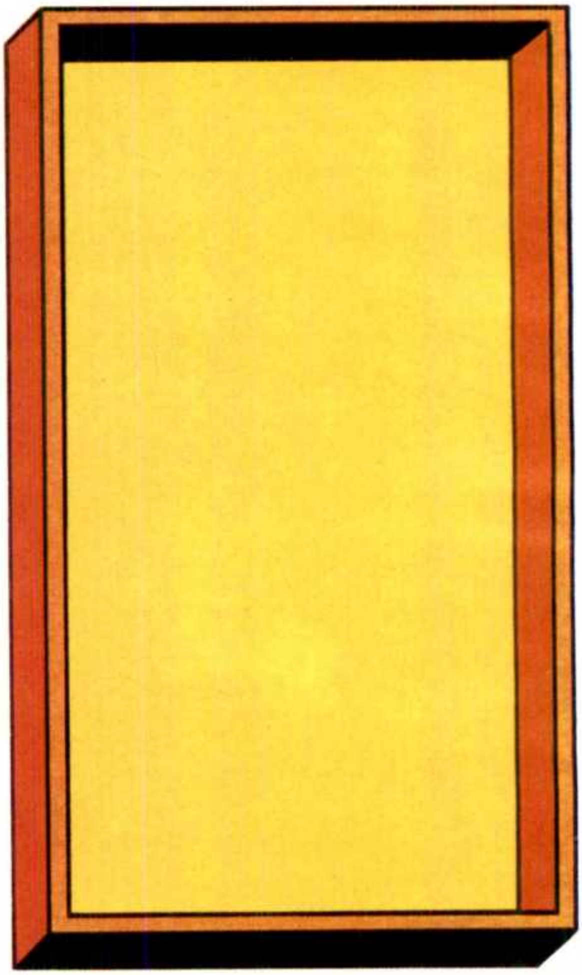
Dithrocream

dithranol BP

Natural selection!



All 40 patients in the trial preferred Dithrocream



Not a single patient preferred dithranol ointment

In a recent clinical trial,[1] Dithrocream 0.25% was compared with dithranol ointment of equivalent strength in the treatment of psoriasis.

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Presentation

Dithrocream 0.1%: pale yellow aqueous cream containing 0.1% Dithranol B.P.
Dithrocream 0.25%: pale yellow aqueous cream containing 0.25% Dithranol B.P.
Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

Indications

For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use

Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications

Acute psoriasis; dithranol sensitivity.

Precautions

Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Basic N.H.S. prices:

Dithrocream 0.1% 50gm tubes £1.81
Dithrocream 0.25% 50gm tubes £1.96
Dithrocream Forte 50gm tubes £2.19

Product Licence Nos.

Dithrocream 0.1%	PL 0173/0029
Dithrocream 0.25%	PL 0173/0028
Dithrocream Forte (0.5%)	PL 0173/0027

References:

1. *British Journal of Dermatology*, 1979, **100**, 475

Full prescribing information is available from
Dermal Laboratories Limited,
Gosmore, Hitchin, Hertfordshire SG4 7QR.

Dithrocream is a registered trade mark.

Now available in three strengths

British Journal of Dermatology (1980) **103**, 1.

Clinical and Laboratory Investigations

Therapy of psoriasis: comparison of photochemotherapy and several variants of phototherapy

H.VAN WEELDEN, E.YOUNG AND J.C.VAN DER LEUN

Institute of Dermatology, State University of Utrecht,
Catharijnesingel 101, Utrecht, The Netherlands

Accepted for publication 3 September 1979

SUMMARY

Four treatments involving light were compared in two series of patients with severe psoriasis. The first series consisted of ten patients, who were treated by the method of paired comparisons. The treatments given were: (1) exposure to fluorescent sunlamps (B); (2) the same, supplemented by fluorescent UV-A lamps (A+B); (3) the same as (2) with the addition of the radiation from germicidal lamps (A+B+C); (4) photochemotherapy with oral 8-MOP followed by UV-A (PUVA); (5) one of the fields served as control, receiving no light at all. The second series consisted of thirty patients. They were treated either with PUVA or with a placebo capsule followed by A+B (pUVAB).

The phototherapies examined differed from many previous attempts in that the increments in dose were made sufficiently large to overcome the increasing tolerance of the skin to light during the treatment.

It is concluded (a) that phototherapy, if conducted in this way, is as effective as PUVA, and (b) that the effectiveness achieved with the phototherapies examined is due to the light from the fluorescent sunlamps (B).

All therapies of psoriasis in which light is used are ultimately based on the experience of many patients, that sunlight has a favourable influence.

The variable intensity and limited availability of sunlight in many parts of the world has led to the use of lamps emitting ultraviolet radiation. The therapies evolved may be classified into two main categories: those with and those without the use of photosensitizers. One recent and successful development in the category with photosensitizers is PUVA therapy (Parrish *et al.*, 1974) where 8-methoxypsoralen (8-MOP) is used as the photosensitizer and UV-A as the radiation. This therapy has become possible by the development of suitable irradiation facilities whereby a total-body irradiation with UV-A can be given in a sufficient dose within a reasonable time. The success of PUVA therapy and the discussions about the possible long-term risks have stimulated many investi-

0007-0963/80/0700-0001 $02.00 © 1980 British Association of Dermatologists

gations, including some on alternative phototherapies without the use of photosensitizers. Experimental indications are promising (Stern & Kihiezak, 1974; Fischer, 1976; Tronnier & Heidbuchel, 1976), but in terms of practical trials, phototherapies have not received the same attention as photochemotherapy.

We have built a light-cabinet with three types of lamps, emitting mainly UV-A, UV-B and UV-C, respectively. Total body exposures can be given with UV-A, such as is used in PUVA therapy, and also with UV-A, UV-B or UV-C separately or in any desired combination, with the proportions variable within wide limits.

MATERIALS AND METHODS

The patients were exposed to UV-radiation of the various wavelength regions in a light cabinet (Fig. 1). It is a square cabinet with inner dimensions 1·50 m × 1·50 m, with reflecting walls and almost fully packed with lamps. The cabinet contains 340 lamps in total. Most of these lamps are of the type Philips TL colour 09, 112 lamps of 40 W and 168 of 20 W; these lamps emit mainly UV-A and are of the same type as used in many cabinets for PUVA therapy. In addition, our cabinet has fluorescent sunlamps of the type Westinghouse FS colour 12, sixteen lamps of 40 W and twenty-four of 20 W; these lamps emit mainly UV-B, with the peak-emission near 310 nm. Furthermore the cabinet has low-pressure mercury arcs of the type Philips TUV, eight lamps of 30 W and twelve of 15 W; such lamps are frequently used as germicidal lamps and emit radiation mainly of wavelength

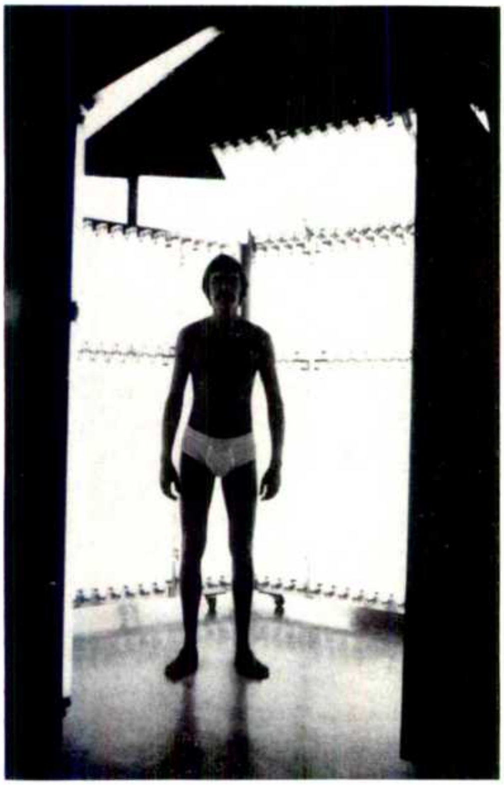

FIGURE 1. Patient in light cabinet.

254 nm in the UV-C. The lamps may be switched on in twenty-eight groups, each group consisting of a small number of lamps (twelve on average) of the same type, distributed symmetrically over the walls of the cabinet. The spectral composition of the UV-radiation in the cabinet may be varied by switching on and off the various groups of lamps.

The irradiances in the various spectral regions were measured with all lamps of a particular type switched on. The spectra of the lamps were not limited strictly to the UV-A, UV-B or UV-C, but the irradiances measured were as well as could be achieved. The measurements were performed with two thermopiles (Hewlett Packard radiant flux detector 8334 A with a window of Suprasil W quartz 1 mm thick, and a Kipp thermopile E 11, with the same window), in combination with various Schott glass cut-off filters to determine the contribution of the different wavelength regions. The Suprasil W quartz window was used as a filter with a complete transmission in the ultraviolet and visible region. The far infrared, however, is cut off by the filter, which makes the thermopiles less sensitive to temperature influences. The irradiance in the UV-A (315–380 nm) was 10·0 mW/cm^2 initially when the lamps were new; it decreased to 6·6 mW/cm^2 during the course of this investigation. The irradiance in the UV-B (280–315 nm) was 0·38 mW/cm^2 and in the UV-C (less than 280 nm) 0·55 mW/cm^2. These latter data are averages over the time of the investigation; the decrease in the irradiance in the UV-B and UV-C lamps was much less than in the UV-A lamps. The smaller decrease in radiant output of the UV-B and UV-C lamps with time may well have rather irrelevant causes: the UV-C lamps were used much less than the other lamps and the UV-B lamps were not new even at the start of this investigation.

In the first investigation, the various therapies were compared in ten patients by the method of paired comparisons (Siemens, 1942, 1943). The patients were selected for widespread symmetrical psoriasis and were over 35 years of age. These patients were hospitalized throughout the investigation. They all had chronic stable widespread plaque type psoriasis vulgaris and had been treated before with various types of coal tar, dithranol and topical steroids. Some of them had also been treated with methotrexate. In each patient six large body-areas received different treatments (Fig. 2). The entire skin, including the control fields, was given 1% salicylic acid in petrolatum, in order to remove scales

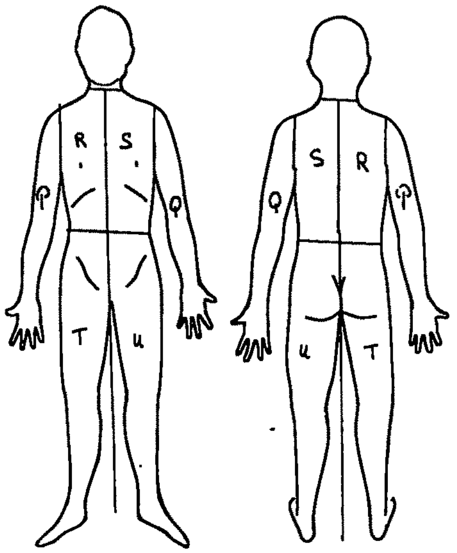

FIGURE 2. Distribution of the skin fields compared.

and to prevent dryness of the skin, which is a common effect of regular exposures to ultraviolet radiation. The salicylic acid was applied after the exposure; in this way it was not likely that the ointment had a specific effect on the various irradiations.

The therapies compared were all given twice a week.

Therapy B: UV-B was given with the aim of eliciting a slight erythema or a slight burning sensation after each exposure. The first exposure given was 70% of the predetermined MED (minimal erythema dose). The successive doses were increased on the basis of day by day observations; if the previous exposure had caused no noticeable effect, the exposure time was increased by 40%; if the previous exposure had caused a slight erythema, the exposure time was increased by 20%; if it had caused more than a slight erythema, the same exposure time was chosen again.

Therapy A+B: UV-A and UV-B were given combined; all the UV-A lamps were used and the same number of UV-B lamps as in therapy B. The guideline for the increments in exposure time was the same as with UV-B.

Therapy A+B+C: UV-A, UV-B and UV-C were given combined; all the UV-A and UV-C lamps were used and the same number of UV-B lamps as in therapy B. The criteria for increasing the dose were the same as in therapies B and A+B.

PUVA therapy: After the other exposures had been completed, the patient was given 8-methoxypsoralen (8-MOP) orally. In the initial phase, patients with body weight up to 60 kg received 30 mg of 8-MOP, with body weight between 60 and 80 kg, 40 mg and with body weight over 80 kg, 50 mg of 8-MOP. At the time the skin had reached its maximum sensitivity (3 h) the skin field selected was exposed in the cabinet with all UV-A lamps switched on; the dose of UV-A was aimed at causing a slight redness. In our hands the maximum sensitivity was reached 3 h after the intake of the psoralens, rather than the 2 h usually quoted by others.

The first exposure amounted to 70% of the MPD (the pre-determined minimal phototoxity dose). The next exposures were given on the basis of day by day observations; if the previous exposure had caused no noticeable effect, the exposure time was increased by 40%; if the previous exposure had caused a doubtful effect, it was increased by 20%; if it had caused a slight erythema, the same exposure time was chosen again; in those few instances where there was more than a slight erythema, the time was reduced by 10% or 20%. When the exposure time reached the limit of 30 min, the doses of 8-MOP were increased gradually, to a maximum of 1 mg/kg body weight. The limit of 30 min in exposure time was chosen because it was thought to be the upper limit the average patient could stand. Initially, the exposure of 30 min amounted to 18 J/cm^2 when the lamps were new; it decreased to about 12 J/cm^2 during the course of the investigation. The slight differences in the increments in exposure times for the phototherapy and the photochemotherapy are based on the difference in the development time of the erythemas and on the difference in the time for the skin to develop tolerance to various treatments (van Weelden & van der Leun, 1976).

Control: as a control, one of the skin fields received no radiation at all.

The effect of the various therapeutic regimes was assessed by experienced clinical observers who had no knowledge about the distribution of the different therapies over the six skin fields (Young, 1970). They made the comparisons only between symmetrically situated skin fields, using the criteria redness, scaling, infiltration and extent.

Most therapies mentioned were represented at least once in each patient. It was arranged so that on each patient there was a comparison between PUVA and A+B, the variant of phototherapy that was thought to be the most promising. Otherwise, the distribution of the therapies over the six skin fields was randomized, and differed from patient to patient. This system allowed for three comparisons per patient, or with ten patients for a total of thirty comparisons.

The second investigation served the same purpose, but the comparison was restricted to two therapies, both applied over the entire skin. In this investigation, too, we had the restriction that the patients were over 35 years of age and were selected for severe psoriasis; symmetry of the lesions was not required. In this series also the patients suffered from chronic stable widespread plaque type psoriasis vulgaris. They had been treated before with different types of coal tar, dithranol, topical steroids and some also with methotrexate (40%). These patients were not hospitalized, but came in twice a week for treatment. The number of patients taking part in this investigation was thirty. On entering the investigation they were assigned strictly alternately to one of the two treatments to be compared. The two treatments were:

PUVA: the treatment was given as described above, but now the entire skin was exposed to the UV-A.

pUVAB: the patients received placebo capsules similar to the 8-MOP capsules, but without the active ingredient. These patients had to wait exactly the same time as the patients in the PUVA regimen before they were exposed to UV-A and UV-B combined, according to the directions for the therapy A+B described above.

The variant of A+B of phototherapy was chosen not only because it was thought to be the most promising variant, but also because the irradiation with the many UV-A lamps switched on was very similar in appearance to the irradiation in the PUVA-therapy.

The patients in both treatments followed the procedure for PUVA therapy. All patients were kept indoors until 6 h had elapsed since the intake of the capsules or till sundown. The patients were informed that it was an experimental treatment, intended to find out how psoriasis could best be treated. They were also informed about the possible risks involved. They were not aware, however, of the differences between the two therapies in case such knowledge might influence the observers' assessments.

The treatments were continued until satisfactory success was achieved, or a more or less constant degree of partial success. If no improvement was apparent after about ten treatments, the patient was given a different treatment; either the other therapy used in this investigation or an entirely different therapy. The results were regularly assessed by experienced clinical observers; the observers knew which two therapies were being compared, but had no knowledge of which was given to any particular patient. The assessments were condensed to the degree of improvement ultimately achieved, again according to the criteria redness, scaling, infiltration and extent, and the number of treatments required.

RESULTS

In the patients assessed by means of paired comparisons P was compared with Q, R with S and T with U (Fig. 2).

The treatments were continued on a patient until all three comparisons had yielded a conclusion; the conclusion 'no difference' was drawn when after ten treatments there was still no difference or if both fields had improved to a satisfactory condition without difference in speed. When the experiment had been completed, the clinical observers decided, by the results of their comparisons, what treatment would be applied to the entire skin. In one patient the investigation had to be discontinued because of a generalized symmetrical eruption, which consisted of redness, papules and scaling. This was probably caused by the 8-MOP, certainly not by the combination of 8-MOP and light. Afterwards the lesions of this patient were cleared by treatment with UV-B. So there remained nine patients and a total of twenty-seven comparisons. Nine of these comparisons formed the main part of this investigation. In three cases, PUVA gave a better result than A+B, in three cases A+B was better than PUVA and in the remaining three cases there was no difference between PUVA and A+B. Thus on average PUVA and A+B were fully equivalent in this comparison.

Nine comparisons were made between the various variants of phototherapy without sensitizers. In four of these, A + B was compared with B, with no clear-cut differences between the two therapies. Three of these comparisons dealt with A + B + C versus A + B; A + B gave a better result in all three cases. Two comparisons were made between A + B + C and B; in both cases B gave the better result.

There were also nine comparisons in which a control field was involved. In four cases the control field was compared with B, in two cases with A + B and in three cases with A + B + C. In all of these nine cases the treatment with 'light' gave better results than the control treatment. It is noteworthy that the control fields themselves generally showed a gradual improvement in the course of the investigation. After the patients had been cleared with the therapy that turned out to be the best during the investigation, the treatment was discontinued. The period before a recurrence of psoriasis differed between the individual patients, but there was no difference in relapse rate between the different skin areas, treated with different therapies during the investigation.

In the second series, where the patients each received one therapy over the entire skin, fifteen patients were given the PUVA therapy and fifteen the therapy designated as pUVAB. In four of these thirty patients the therapy could not be completed because of different complications. The pUVAB therapy had to be stopped in one patient, the PUVA therapy in three cases. In the remaining patients, twelve on PUVA and fourteen on pUVAB, the results noted in regular assessments by the observers were condensed into the final improvement achieved and the number of treatments required for this result. The light doses given to patients in this series are shown in Table 1.

TABLE 1. Light doses given to patients

Type of therapy	Average initial dose	Average final dose
PUVA (the dose of psoralens increased to a maximum of 1 mg per kg body-weight)	5·7 ± 2·3 J/cm^2 UV-A (315–380 nm)	14·4 ± 1·6 J/cm^2 UV-A (315–380 nm)
pUVAB	162 ± 82 mJ/cm^2 UV-B (280–315 nm) combined with 3·5–1·8 J/cm^2 UV-A	2416 ± 693 mJ/cm^2 UV-B (280–315 nm) combined with 13·2–3·8 J/cm^2 UV-A

On both types of therapy most of the patients improved very well, according to the criteria redness, scaling, infiltration and extent. The number of exposures required to show improvement of 80% or more, relative to the pretreated state, was for the PUVA regimen 25 ± 5 and for the pUVAB treatment 28 ± 6. In these out-patients there was again no significant difference between photochemotherapy and phototherapy.

In this series too, the therapy was discontinued when the psoriasis was cleared or had improved to a satisfactory level. The time before a recurrence of the psoriasis, i.e. when the patients needed treatment again, was 5·2 ± 2·8 months for the PUVA treatment and 5·4 ± 4·3 months for the pUVAB regimen.

DISCUSSION

In both series of patients, where the comparisons were made between PUVA and A + B, there was no significant difference in effectiveness. The number of exposures required for a clearance of 80% or more was 27 ± 5 with PUVA and 28 ± 6 with the pUVAB treatment; these numbers do not show a statistically significant difference ($P > 0·05$, Student's t-test).

The method of paired comparisons in the same patient and at the same time is very efficient and is more convincing than the method of comparing two therapies in different patients. On average PUVA and A+B were fully equivalent in this comparison. If there had been a clear-cut difference in effectiveness between PUVA and A+B, nine comparisons should have been sufficient to establish that with statistical significance. There was, however, not even a slight indication of such a difference. Nevertheless, it should be stressed that the assessments given by the observers did suggest real differences in the therapeutic effect of PUVA and A+B in individual patients. Overall these differences cancelled out each other. The possibility that the differences in effectiveness between PUVA and A+B observed in individual patients were found just by chance is not supported by the comparisons between the variants of phototherapy without sensitizers. In no patient was there an observable difference in result between the therapies B and A+B. The addition of all the 280 UV-A lamps did not change the therapeutic results and meant only an extra heat-load for the patients. In all patients therapy A+B+C produced less result than either therapy B or A+B. The addition of UV-C limited the exposure by its strong ability to cause erythema, which was apparently not matched by a comparable therapeutic effectiveness, and so resulted in a negative effect. In the same way it is conceivable that greater effectiveness of the therapy with UV-B could be achieved by filtering out the shortest wavelengths emitted by the fluorescent sunlamps. In that respect one should keep in mind the results of Fischer (1976), Stern & Kihiezak (1974), Tronnier & Heidbuchel (1976) and at the Dead Sea which all point in a similar direction.

All variants of phototherapy, even A+B+C, worked better than the control. Even the control field improved to some extent. It can not be established whether the gradual improvement of the psoriasis on the control field had anything to do with the irradiation of the other parts of the body; it may just as well have been caused by the salicylic acid in petrolatum, which was applied over the entire skin, or to some other favourable influence connected with the hospitalization (Siemens, 1954).

The over-all conclusion is that the order of effectiveness is: (1) PUVA = A+B = B; (2) A+B+C; (3) control. Phototherapy, if conducted properly, in general is as effective as PUVA in clearing psoriasis. It is also concluded that the effectiveness achieved with the phototherapy is due to the light from the sunlamps.

Not only was there no difference in clearing the psoriasis, but also in the remission period there turned out to be no significant difference between PUVA (5·2±2·8 months) and pUVAB (5·4±4·3 months) ($P > 0·05$; testing the null hypothesis using the Student's '*t*'-distribution). This shows a remarkable parallelism with the remission period found by Avrach (1979) after climatotherapy of psoriasis at the Dead Sea, Israel. He stated that the average period before a recurrence of psoriasis was 5·6 months in patients returning for a second treatment session. Cripps & Lowe (1979) reported a similar initial remission time, average 5 months, after clearing patients with PUVA. It appears that the various types of therapy discussed here do not produce any appreciable differences in remission time. The choice between photochemotherapy and phototherapy will, therefore, depend not on the effectiveness or on remission time, but on side-effects, economics and so on. Phototherapy has practical advantages. For the patients it is less time-consuming and gives a smaller heat-load during the exposure. It makes the patient not more, but less, sensitive to sunlight. It does not cause as many side-effects as are known from photochemotherapy. Phototherapy is more economical than PUVA; fewer lamps are needed, less energy is consumed and the exposure times are shorter.

Both photochemotherapy and phototherapy are potentially carcinogenic. Sufficient hard data are not available at the present for making a comparison. The risks of phototherapy in this respect are at least better known, because of the vast experience with sunlight and artificial sources of ultraviolet radiation. In countries of N.W. Europe only a small proportion of people ever get solar carcinomas and those are almost invariably people who have spent a long life outdoors. A series of treatments with ultraviolet radiation from artificial sources must be rather active and prolonged to come anywhere near such a dose. Furthermore, there are some indications that photochemotherapy might be more carcinogenic that phototherapy. According to Baden *et al.* (1972) the amount of repair DNA synthesis relative to the degree of inhibition of scheduled DNA synthesis in cultured human fibroblasts is much less for 8-MOP and long wave ultraviolet-treated cells than for those exposed to short wave ultraviolet.

Also Griffin, Hakim & Knox (1958) showed that with just about the same acute effects the combination of 8-MOP and long-wave ultraviolet radiation is more carcinogenic in mice than short-wave ultraviolet.

In view of these arguments it is advisable to start treating patients with UV-B instead of PUVA. In those cases where the UV-B therapy turns out to be ineffective one can try PUVA as an alternative because in some patients, as shown by our investigation, PUVA appears to produce a better result, although the opposite also occurs.

To be effective, phototherapy requires that the tolerance which the skin develops is overcome. This is perhaps one of the reasons why phototherapy was not very effective in earlier experiments (Young, 1972).

The experiences within our department of photobiology with light-induced tolerance in photodermatoses and normal skin for several years now have been of much help in that respect. Phototherapy, with two irradiations a week, requires an average dose-increase of 20% per session during the initial phase of the treatment. At the end the dose increase is less steep, because the tolerance which the skin has built up is approaching a more or less constant level. The dose increase with phototherapy is more than with PUVA (10% on average in the initial phase), but even in the long run there is no problem in achieving that increment with UV-B cabinets of appreciably smaller power than those used for PUVA-treatment.

We do prefer to discontinue the treatment as soon as the psoriasis is cleared. The psoriasis will usually stay in remission for several months. This has the advantage that as soon as the patient needs treatment again, the tolerance induced has decreased or even completely disappeared. In this way, the total dose of UV-radiation administered is limited; firstly by not giving any exposures during the remission period and, secondly, by the fact that smaller doses will suffice when the therapy has to be resumed.

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Long-term photochemotherapy: histopathological and immunofluorescence observations in 243 patients

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SUMMARY

Skin biopsies of 243 patients treated with photochemotherapy (PUVA) for 1–4 years were examined histologically. Two hundred and six patients were examined retrospectively after total cumulative UV-A doses of 579·6±598·0 J/cm^2 (mean±s.d.). An eosinophilic homogenization and a reduction of elastic fibres at the dermo–epidermal junction, and an increase of dermal macrophages were found as possible abnormalities. However, except for the increase of melanophages there was no statistically significant correlation between the incidence of these changes, the total UV-A dose applied and the skin type of the patients. Neither were such correlations found in thirty-seven patients biopsied twice after 394·8±267·6 J/cm^2 and 808·5±458·9 J/cm^2 (mean±s.d.), respectively. Studies with direct immunofluorescence techniques revealed no immunoglobulin deposits in PUVA treated skin in fifty-six patients after 469·2±370·2 J/cm^2; antinuclear antibodies were observed in 4·6% of 129 patients after 169 J/cm^2 (mean); in 11% of fifty-three patients reexamined after 381 J/cm^2 (mean) and in 13·6% of twenty-two patients reexamined a second time after 643 J/cm^2 (mean).

Thirteen out of a total of 572 patients developed a peculiar mottling of skin in areas previously overdosed by PUVA. Subepidermal homogenization and reduction of elastic fibres were found in 45% of the patients, indicating that these changes indeed are a consequence of PUVA. Nuclear and cellular irregularities were found in 45% of the biopsies and 63% showed a disturbed epidermal architecture, but no carcinomas were observed. PUVA-induced mottling was reversible in 31%, partially reversible in 15%, but continued to be present in 54%.

There are concerns that 8-methoxypsoralen-photochemotherapy (PUVA) (Parrish *et al.*, 1974; Wolff *et al.*, 1976) may lead to late side-effects, notably degenerative changes and cancer of the skin (Urbach, Forbes & Davies, 1976; Wolff *et al.*, 1977a; Gschnait, 1977; Fitzpatrick *et al.*, 1977). Though we are aware that only long-term studies spanning perhaps a decade or more may eventually substantiate or

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invalidate such concerns, we recognize that interim reports on long-term PUVA patients, examined specifically with regard to this issue, may assist in decisions concerning the initiation or continuation of this treatment in patients with psoriasis. Indeed, recent studies have shown that there is an increased risk of long-term PUVA patients to develop non-melanoma skin tumours if they are highly light sensitive or have a history of pre-PUVA skin cancer, ionizing irradiation (Stern *et al.*, 1979) or arsenic therapy (Hofmann, Plewig & Braun-Falco, 1979; Hönigsmann *et al.*, 1979b). We now wish to report on a histopathological follow-up of 243 psoriatics, who had been under continuous PUVA treatment for periods ranging from 1 to 4 years.

MATERIALS AND METHODS

The 243 patients studied were selected from a series of 572 patients who had been treated solely with PUVA at our institutions since 1974. All patients had had widespread, severe psoriasis before PUVA treatment and had been treated previously with topical corticosteroids, tar or dithranol and UV light. Forty-one per cent of the patients had previously had either one or more of the following treatments: arsenic, methotrexate, X-ray or Grenz ray therapy. The patients were treated with PUVA according to established methods (Wolff *et al.*, 1977b) and during this treatment none of them received any other systemic or topical treatment except oil baths and emollients. Before every treatment session, the patients were seen by an experienced dermatologist and treatment results, side-effects and course were carefully documented. After complete clearing the patients were kept on maintenance treatment, which initially consisted of three irradiations per week and was gradually reduced to one irradiation every 3 weeks or once a month as described previously (Wolff *et al.*, 1977b). The frequency of maintenance treatments was monitored according to the disease activity and frequency or severity of recurrences. The goal was to keep the patients continuously free of psoriatic lesions by a minimum of maintenance treatment sessions. Therefore, maintenance treatment was frequently discontinued when the patients had been free of psoriasis for periods longer than 3 months and was only resumed when a recurrence was observed. Results of such treatment have been published (Wolff *et al.*, 1976, 1977a; Gschnait, 1977).

8-Methoxypsoralen (8-MOP) was employed in 10 mg capsules (Oxsoralen, Gerot Pharmaceuticals, Vienna), PUVA 4000, 6000 and 6001 irradiation units (Waldmann, Schwenningen, FRG) served as UV-A sources. The emission spectrum of these units has been detailed elsewhere (Wolff *et al.*, 1976). The UV-A output of the irradiation units was measured with a cosine corrected photometer (Ultraviolet Products, St Gabriel, California) and found between 5 and 10 mW/cm^2 depending on the age of the light bulbs.

Histopathological study

Group 1. Two hundred and six patients (mean age 46·9 ± 16·7 years) were investigated retrospectively. The patients were assigned to different groups according to the total amount of UV-A energy received during photochemotherapy and sub-groups were established according to skin type. Punch biopsies were obtained from buttock skin, previously not involved with psoriasis. The mean total UV-A dose for the whole group of patients was 579·6 ± 598·0 J/cm^2 (mean ± s.d.), the highest dose being 4150 J/cm^2.

Group 2. Thirty-seven patients were biopsied twice during PUVA treatment. Biopsies were again taken from previously non-involved buttock skin. The mean total dose of UV-A at the time of the first biopsy was 394·8 ± 267·6 J/cm^2 and 808·5 ± 458·9 J/cm^2 at the time of the second biopsy.

Biopsy material was embedded in paraffin and stained with haematoxylin and eosin and with Weigert's elastica stain.

Immunofluorescence (IF) study

Direct immunofluorescence. Biopsies taken from previously uninvolved buttock skin of fifty-six patients were processed for direct immunofluorescence. Specimens were snap frozen in liquid nitrogen and stored at −20°C until examined. Cryostat sections (6 μm) were incubated with FITC conjugated polyvalent anti-human antiserum, IgG and C3 antiserum (Hyland), all of goat origin. Direct immunofluorescence was performed according to established methods. The mean total UV-A dose in this group of patients at the time of biopsy was $469{\cdot}2 \pm 370{\cdot}2$ J/cm^2.

Antinuclear antibodies. One hundred and twenty-nine patients were examined for the presence of antinuclear antibodies at various intervals after initiating and during PUVA treatment. Cryostat sections of rhesus monkey oesophagus and rat liver were used as substrates and a commercial FITC conjugated goat anti-human IgG-antiserum (Hyland) was used as indicator reagent. Staining was performed according to established methods.

PUVA induced mottling of the skin

Out of a total of 572 patients treated and continuously followed up at our institutions, thirteen had developed mottled, hyper- and hypo-pigmented changes in different regions of the body (for clinical details, see the Results section). These patients were investigated separately and biopsies were taken from both hyper- and hypopigmented skin and processed for routine histopathology (hematoxylin and eosin, and elastic tissue stains) and epidermal sheets prepared with the sodium bromide technique were incubated for the histochemical DOPA reaction.

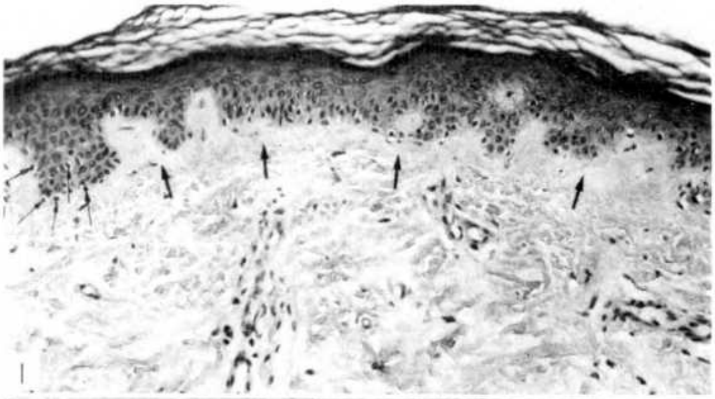
RESULTS

Histopathology

Group 1

At low magnifications, the epidermis and dermis of practically all specimens appeared essentially normal and there was no evidence of psoriasis. There was a varying amount of hyperkeratosis which tended to be less in patients who had been off treatment for more than 3 weeks or were kept on a low frequency (once per 2–4 weeks) maintenance therapy regimen. Occasionally, there was thinning of the epidermis and flattening of the papillary body and this was found mostly in older patients. There was an obvious increase in the number of melanocytes and in the amount of melanin in the epidermis of all specimens. Melanin granules were found not only in the basal layer but were present high up in the epidermis and could even be recognized within the stratum corneum.

Apart from these findings, the specimens did not exhibit changes that could readily be identified as pathological. Careful scrutiny of the sections, however, suggested three types of changes as possibly abnormal: (1) a thin band of eosinophilic homogenization at the dermo–epidermal junction (Fig. 1). As shown in Table 1, subepidermal homogenization was observed in thirty-two out of 206 cases (15·5%); (2) a reduction of elastic fibres within this zone (Fig. 2) which was observed in twenty-five out of 206 cases (12%) (Table 1); and (3) an increase of the number of dermal melanophages, observed in forty-one out of 206 cases (20%). Cellular pleomorphism similar to that recently described under the term 'epidermal dystrophy' (Cox & Abel, 1971) was not observed.



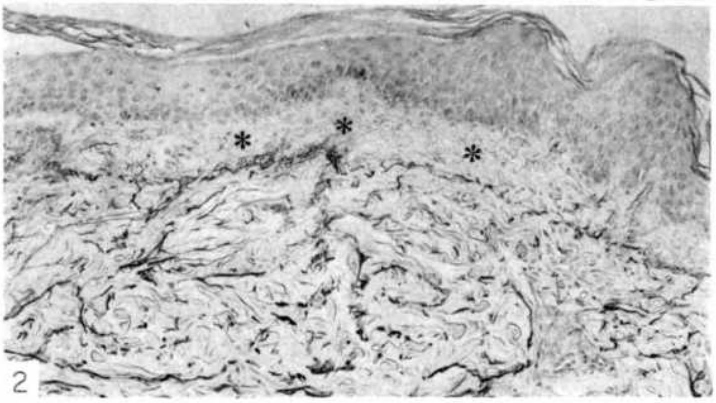


FIGURE 1. Long-term PUVA treated skin, demonstrating hyperkeratosis and increased numbers of melanocytes (at the left side of the figure these are marked by fine arrows). At the dermo-epidermal junction there is a thin band of eosinophilic homogenization (thick arrows) (H & E, × 130).

FIGURE 2. Reduction of elastic fibres within the subepidermal zone (asterisks) (Elastica stain, × 130).

TABLE 1. Histopathological follow-up. Two hundred and six patients, total UV-A dose 579 ± 598 J/cm², max. 4150 J/cm², duration of treatment 1–4 years

Total UV-A dose (J/cm²)	Subepidermal homogenization	Reduction of subepidermal elastic fibres	Increased numbers of melanophages
< 200	9/44 (20·4%)	3/44 (6·8%)	0/44 (0%)
200–400	5/56 (8·9%)	6/56 (10·7%)	8/56 (14·2%)
400–600	5/34 (14·7%)	2/34 (5·8%)	8/34 (23·5%)
600–800	3/26 (11·5%)	4/26 (15·3%)	10/26 (38·4%)
800–1000	5/20 (25·0%)	5/20 (25·0%)	5/20 (25·0%)
> 1000	5/26 (19·2%)	5/26 (19·2%)	10/26 (38·5%)
Overall	32/206 (15·5%)	25/206 (12·1%)	41/206 (19·9%)

Random distribution of s.h. and r.e.f. in the different groups ($\chi^2 = 4·6$ and 7·11, respectively; not significant).

Significant increase of melanophages ($\chi^2 = 23·7$; $P < 0·05$).

TABLE 2. Dermal pathology, skin type and UV-A energy delivered

Total UV-A dose (J/cm²)	Subepidermal homogenization	Reduction of subepidermal elastic fibres	Increased numbers of melanophages
Skin type I/II patients			
<200	3/14 (21·4%)	2/14 (14·2%)	0/14 (0%)
200–600	2/16 (12·5%)	1/16 (6·2%)	1/16 (6·2%)
>600	3/10 (30%)	3/10 (30%)	3/10 (30%)
χ^2	1·2	2·7	6·3
P	0·6	0·3	<0·05
Skin type III/IV patients			
<200	5/33 (15·1%)	3/33 (9·0%)	0/33 (0%)
200–600	8/71 (11·2%)	5/71 (7·0%)	14/71 (19·7%)
>600	10/62 (16·1%)	10/62 (17·7%)	21/62 (35·4%)
χ^2	0·9	3·2	13·2
P	0·7	0·2	<0·005

Correlation between pathology and the total amount of UV-A energy delivered (*Table* 1). The incidence of subepidermal homogenization was distributed randomly in the different groups ($\chi^2 = 4{\cdot}6$; statistically not significant). There was no correlation between the UV-A dose applied and the incidence of the reduction of subepidermal elastic fibres ($\chi^2 = 7{\cdot}11$, statistically not significant) but the incidence of increased numbers of dermal melanophages showed a statistically significant correlation with the amount of UV-A energy delivered ($\chi^2 = 23{\cdot}7$; $P<0{\cdot}05$) (Table 1).

Correlation between dermal pathology, skin type and the amount of UV-A energy delivered (*Table* 2). When patients were assigned to different groups according to their skin type and the amount of UV-A energy delivered during PUVA treatment there was again a random distribution of the incidence of subepidermal homogenization and a reduction of subepidermal elastic fibres within the different groups ($\chi^2 = 0{\cdot}9$–$3{\cdot}2$) and the differences were therefore statistically not significant. A significant correlation was found between the increased numbers of dermal melanophages and the total UV-A dose applied both in skin type I/II ($\chi^2 = 6{\cdot}3$; $P<0{\cdot}05$) and in skin type III/IV ($\chi^2 = 13{\cdot}2$; $P<0{\cdot}005$) patients.

Group 2

Similar alterations were found in the patients who had been biopsied twice. The total mean dose of UV-A of this patient group was 394·8±267·6 J/cm² at the time of the first biopsy and 808·5±458·9 J/cm² at the time when the second biopsy was taken. Subepidermal homogenization was found in nine out of thirty-seven (24·3%) of the patients at the time of the first biopsy but only in five out of thirty-seven (13·5%) of the patients when the second biopsy was taken (Table 3). On the other hand, there were differences in neither the subepidermal elastic fibre reduction nor the numbers of dermal melanophages between the first and second biopsies (Table 3). Specimens obtained from the normal appearing skin of a 62-year-old patient (334 J/cm² at the first biopsy, 1138 J/cm² at the second biopsy) showed an irregular epidermis with loss of palisading of the basal cell layer, nuclear pleomorphism and dyskeratotic cells (Fig. 3). The history of this patient revealed that he had previously been repeatedly on treatment regimens with arsenic, 15–20 years prior to the initiation of PUVA therapy.

TABLE 3. Histopathological follow-up of thirty-seven patients, biopsied twice

	Subepidermal homogenization	Reduction of subepidermal elastic fibres	Increased numbers of melanophages
1st biopsy ($394 \cdot 8 \pm 267 \cdot 6$ J/cm^2)	9/37 (24·3%)	4/37 (10·8%)	10/37 (27·0%)
2nd biopsy ($808 \cdot 5 \pm 458 \cdot 9$ J/cm^2)	5/37 (13·5%)	4/37 (10·8%)	13/37 (35·1%)

Immunofluorescence study

Direct IF. In fifty-two out of fifty-six (92·8%) of the specimens, no immunoglobulin deposits were found in the skin. One patient exhibited IgM deposits in the horny layer. In two specimens, an irregular patchy fluorescence of the dermo–epidermal junction was observed when polyvalent anti-human globulin and anti-IgG antisera were employed for staining. One patient showed IgG and IgM cytoid bodies at the dermo–epidermal junction.

Antinuclear antibodies (*Table 4*). Six out of 129 patients (4·6%) examined $3 \cdot 5 \pm 4 \cdot 0$ (mean ± s.d.) months after initiation of PUVA therapy and after having received $159 \cdot 9 \pm 193 \cdot 3$ J/cm^2 showed a positive ANA test. Among fifty-three patients of this group who were re-examined $12 \cdot 1 \pm 4 \cdot 2$ months

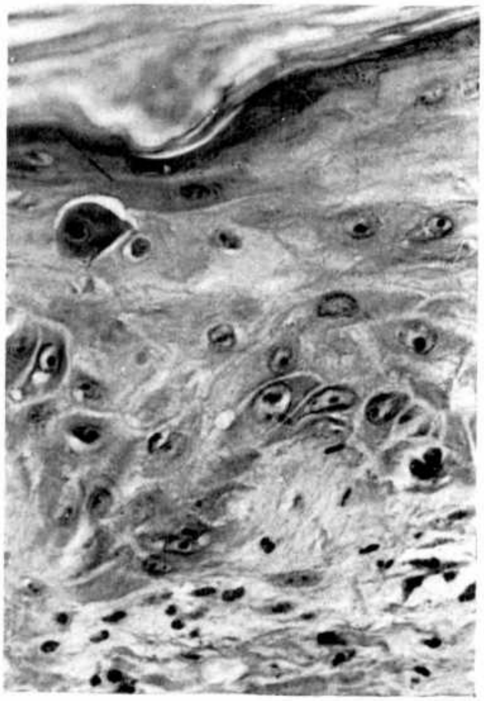


FIGURE 3. Biopsy from long term PUVA treated skin of a psoriatic patient, who had repeatedly been on arsenic. Note the pleomorphism of nuclei and the dyskeratotic cell (H & E, × 340).

TABLE 4. ANA in psoriatics treated with PUVA

	Duration of PUVA treatment (months)	Total UV-A dose (J/cm^2)	Number of positive results
1st examination	3·5 ± 4·0	159·9 ± 193·3	6/129
2nd examination	12·1 ± 4·2	381·0 ± 284·5	6/53*
3rd examination	23·6 ± 6·6	643·6 ± 401·0	3/22†

* 3/53 had converted to positive, 2/53 to negative.

† 1/22 had converted to positive, 1/22 to negative, when compared to the second examination.

after initiation of PUVA and after having received a total UV-A energy dose of 381·1 ± 284·5 J/cm^2, there were again six (11%) who exhibited a positive ANA test. When the results of the ANA positive patients were compared to their first examinations it was found that three of fifty-three patients, in their second test, had converted to ANA positive, whereas two out of fifty-three who were positive at the time of the first examination were now negative. A third ANA test was performed in twenty-two patients 23·6 ± 6·6 months after the first PUVA exposure and after a total UV-A dose of 643·6 ± 401·0 J/cm^2. Three out of twenty-two (13·6%) showed a positive ANA on this occasion. When the results of the third ANA test were compared to the second ANA test, it became evident that one patient had converted to positive whereas one had become negative. The ANA titres of all ANA positive patients were low (1:10 to 1:40) and those patients who exhibited a positive ANA test on more than one occasion during PUVA treatment failed to show an increase in ANA titre.

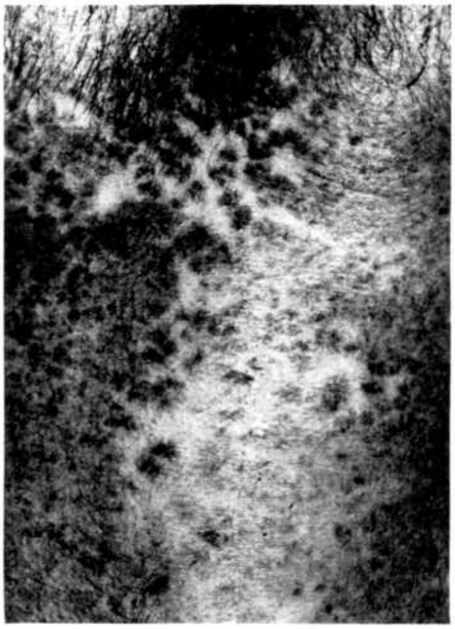


FIGURE 4. Mottled skin of a 48-year-old patient. First appearance of mottling after 425·1 J/cm^2.

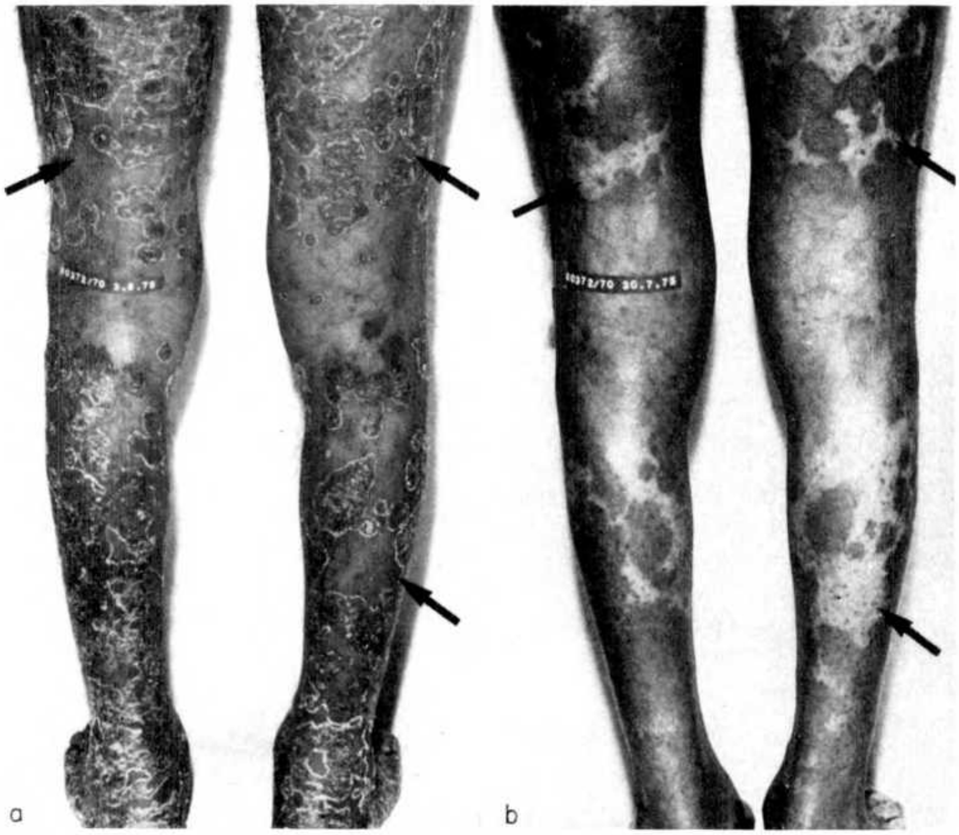


FIGURE 5. (a) Erythema (arrows) due to PUVA-overdosage mainly confined to normal, non-lesional skin. (b) Development of depigmentation and initial mottling in the previously overdosed areas (arrows), approximately 2 months later.

Studies on mottled skin induced by PUVA

Clinical changes. Thirteen out of a group of 572 patients treated with PUVA at our institutions had developed a peculiar mottled skin consisting of irregular hyper- and depigmentation in various regions of the body. In addition, the skin in these regions appeared dry, glistening and somewhat atrophic (Fig. 4). In all patients, the mottling had occurred in skin sites which had been overdosed and had exhibited a + + + to + + + + erythema during the initial treatment phase (Fig. 5) and after clearing had been continuously re-exposed during maintenance treatment. One patient was of skin type I, nine were of skin type II and three of skin type III. Mottling was first noted between 4 weeks and 12 months ($6{\cdot}7 \pm 4{\cdot}3$ months) after the first PUVA exposure and at this time 154·4–1085·4 J/cm^2 (mean $520{\cdot}3 \pm 316{\cdot}3$ J/cm^2) had been applied. At the time of examination, the patients were still receiving maintenance therapy (the total UV-A energy dose being $911{\cdot}4 \pm 848{\cdot}5$ J/cm^2 at the time of follow-up). At this time, four of the thirteen patients had repigmented completely showing an apparently normal skin without evidence of atrophy in the previously mottled areas, two had repigmented partially whereas seven still exhibited the mottled skin (Table 5).

TABLE 5. PUVA induced mottling

Total UV-A energy at follow-up	911 ± 848
Complete repigmentation	4/13 (31%)
Partial repigmentation	2/13 (15%)
No change	7/13 (54%)

Histopathology. Histologically, there was hyperkeratosis, thinning of the epidermis, flattening and in some specimens complete disappearance of the rete ridges. In 63% of the patients, there was some disorganization of the epidermal architecture, loss of palisading of the basal layer and in 45% of the patients some pleomorphism of the epidermal cells and occasional dyskeratotic cells were found (Table 6, Fig. 6). The hyperpigmented areas showed a dramatic increase of melanocytes and heavy pigmentation of the epidermis, whereas in the depigmented areas the numbers of melanocytes and the total amount of epidermal melanin were considerably decreased. The melanocytes were large and appeared irregular exhibiting large hyperchromatic and irregular nuclei and were laden with melanin whereas the surrounding epidermal keratinocytes were practically devoid of melanin pigment. Epidermal sheets subjected to the DOPA stain confirmed that in hyperpigmented areas melanocytes were increased in number, strongly DOPA-positive and highly dendritic; in the depigmented areas

TABLE 6. Histopathology of PUVA induced mottling

	Patients (%)
Disturbed epidermal architecture	63
Nuclear and cellular irregularities	45
Subepidermal homogenization	45
Reduction of elastic fibres	45

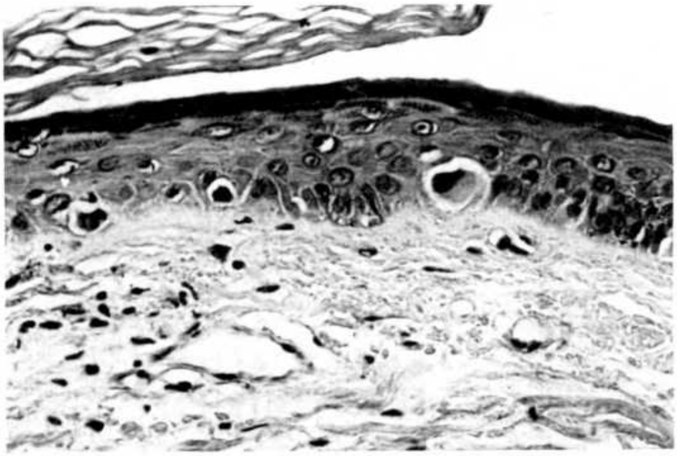


FIGURE 6. Hypopigmented area of mottled skin. Hyperkeratosis and disappearance of the rete ridges. Melanocytes appear irregular, exhibiting atypical nuclei (H & E, × 190).

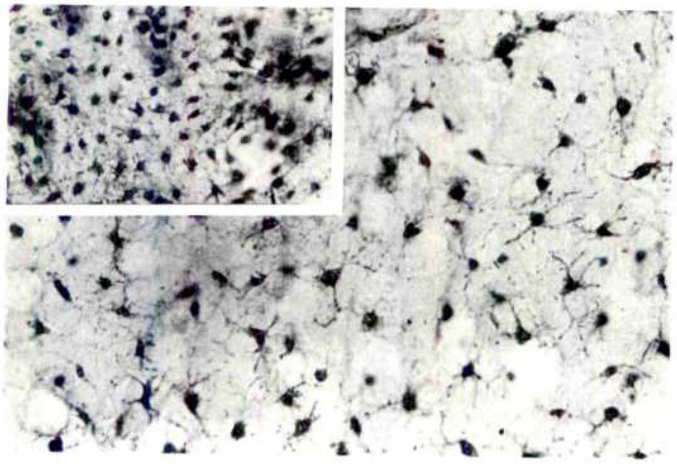


FIGURE 7. Hypopigmented skin. Melanocytes are sparse as compared to non-mottled control skin of same region in same patient (inset). The individual cells are increased in size and show marked elongation and arborization of the dendrites. Control melanocytes in the inset were photographed at the same magnification. DOPA stained epidermal sheet preparations (× 100).

melanocytes were decreased in number but appeared larger and occasionally were of bizarre shape, exhibiting a marked elongation and arborization of dendrites (Fig. 7).

DISCUSSION

In a preliminary study published previously (Wolff *et al.*, 1977a), we were unable to detect frank pathological changes in the skin of patients treated with PUVA for up to 2 years. Others (Braun-Falco, Hofmann & Plewig, 1977), studying short-term histological effects in PUVA treated skin, have also failed to note significant alterations, whereas a third group found focal dystrophy of epidermal cells in 50% of thirty-seven patients treated with PUVA for up to 1 year (Cox & Abel, 1979). The present investigation confirms our original impression that subepidermal homogenization, loss of subepidermal elastic fibres and an increase of dermal melanophages are the only changes which can be consistently detected in the skin of patients treated with PUVA for prolonged periods of time. The question arises whether these alterations herald early degenerative changes induced by PUVA or are due to previous treatments or a result of long lasting psoriasis. Most of our patients had had severe long standing psoriasis and previous treatments with corticosteroids, tar and dithranol and 40% had received either arsenic, grenz rays or methotrexate before PUVA was initiated. The severity of psoriasis in our patients and the fact that PUVA treatment had been given for up to 4 years explains the relatively high UV-A energy load some of our patients have received. At the ultrastructural level we have found that the subepidermal homogenization is mainly due to reduplications of the basal lamina, the deposition of a finely fibrillar material in the sub-epidermal region and a reduction of elastic fibres in the subepidermal regions. Similar findings have been reported by others recently (Hashimoto *et al.*, 1978). Such changes can occur in a host of inflammatory conditions of the dermo–epidermal junction and are therefore not uniquely characteristic for PUVA treated skin. In this study, the incidence of subepidermal homogenization and reduction of elastic fibres correlated with neither the total cumulative dose of UV-A energy given to the patients nor the skin type of

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the patients and this seemingly militates against the suggestion that they are a direct consequence of PUVA treatment; also, in the group of patients who had been biopsied twice the incidence of these changes was lower at the time of the second biopsy. Nonetheless, both the subepidermal homogenization and the reduction of elastic fibres were most frequently seen in the patients who had received the highest amount of UV-A energy and this suggests that these changes may become statistically significant after UV-A doses which are in excess of the mean cumulative doses computed for the present series. The fact that identical changes were observed in patients with PUVA-induced mottling in addition indicates that there is indeed a causal relationship between PUVA and the described histological alterations. We therefore tentatively conclude that, although chronic degenerative changes of the dermis cannot be unequivocally shown to be a direct consequence of PUVA in our patient material, we do expect such alterations to develop after even higher total cumulative doses of PUVA and longer periods of treatment.

In contrast to the histopathological alterations discussed so far, the phenomenon described in this paper as mottling of the skin is unequivocally a direct consequence and thus a chronic side-effect of PUVA treatment. The fact that mottled skin areas are atrophic and show a disturbance of the regular architecture of the epidermis, which is similar to what has been described recently as 'epidermal dystrophy' (Cox & Abel, 1979), indicates that this is not a purely pigmentary phenomenon, but that the entire epidermis and the papillary dermis are involved. Dermal changes again included subepidermal homogenization and destruction of elastic fibres which represents cogent evidence that these alterations are in fact related to PUVA treatment. This phenomenon was observed in nine out of thirteen patients and was clearly related to overdosage during the initial phases of PUVA treatment, namely erythema and blistering. Since, however, mottling only developed after a considerable lag phase during which PUVA treatment had been continued, it appears that both overdosage and subsequent PUVA exposures to these sites jointly contribute to this derangement of skin structure. At least at the clinical level, these changes have proven reversible in four out of thirteen patients, and partially reversible in two, but only future studies will be able to determine the actual course and prognosis of this long-term side-effect. PUVA-induced mottling certainly suggests that overdosage phenomena should be avoided and that, if they occur, the affected areas should be shielded from further PUVA exposures. A PUVA regimen is now available which renders PUVA overdosage highly improbable (Hönigsmann *et al.*, 1979a) and this should prevent PUVA induced mottling altogether.

Although the epidermis of mottled skin did show some structural derangement, it did not exhibit changes suggesting frank or developing actinic keratoses, or bowenoid changes. This correlates with the fact that, clinically, keratoses and tumours have not been observed in PUVA-mottled skin of our patients. In our material of all patients treated with PUVA so far the incidence of basal and squamous cell carcinomas is increased over that expected for the general population of our geographic area only in patients who previously had had treatment with arsenic (Hönigsmann *et al.*, 1979b). This is in line with the results of a series published recently (Stern *et al.*, 1979), where increased skin-tumorigenesis in long-term PUVA patients was correlated with a previous history of skin cancer, high sensitivity to sunlight and a history of pre-PUVA ionizing radiation therapy. The one patient in the present series whose biopsy of clinically normal appearing skin had shown cellular pleomorphism of the epidermis (Fig. 3) had also had arsenic treatment years before PUVA was initiated, and this parallels the observations of Braun-Falco *et al.* (1977) and the more recent results of Hofmann *et al.* (1979) who found bowenoid changes in clinically normal appearing skin of long-term PUVA patients who previously had had arsenic therapy. In view of the fact that 16·8% of our patients had a history of arsenic intake, it is surprising that histologically detectable cellular pleomorphism and bowenoid changes were not found more often, but since such changes tend to be focal—as is also the case in early, histologically detectable actinic malignancy of the epidermis (Pearse & Marks, 1977)—and, since multiple biopsies were not done in the same patients at the same time, it is possible that focal dysplastic epidermal changes were missed in some of our patients. Thus, it is evident that despite the apparent absence of histologically detectable malignant changes in the present patient material a previous exposure to arsenic bears a definite carcinogenic risk for patients treated with PUVA; as will be detailed elsewhere (Hönigsmann *et al.*, 1979b) arsenic is another risk factor to be added to those already listed by Stern *et al.* (1979) namely previous history of carcinoma, ionizing irradiation and light sensitivity, and that arsenic may therefore be one reason to exclude a patient from PUVA treatment.

Our study shows that PUVA does not lead to the deposition of immunoglobulins or immune complexes in the skin. Neither does it lead to a significant formation of anti-nuclear antibodies which suggests that possible PUVA-induced alterations of nuclear material are not sufficiently antigenic to elicit antibody formation. Our results contrast the observation of other authors (Bjellerup *et al.*, 1979) who, albeit in a smaller series of patients, did find an increased incidence of ANA in PUVA treated psoriatics. This discrepancy remains to be clarified.

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Decreased epidermal aryl hydrocarbon hydroxylase and localized pustular psoriasis

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SUMMARY

Microsomal aryl hydrocarbon hydroxylase (AHH) activity was measured in suction separated epidermis from forearm skin of thirteen patients with localized palmo–plantar pustular psoriasis and thirteen normal subjects before and after induction by benzanthracene. AHH activity had a mean value of 2·32 μm 3OH-BP/mg microsomal protein/h ± 0·23 (s.e.) in the patients and 3·41 ± 0·23 in the normal subjects. AHH induction was also decreased with a mean value of 1·15 ± 0·1 (s.e.) compared with 1·98 ± 0·14 for the normal subjects. Since in twelve of the patients the psoriasis had always been localized to the palms and soles, the decreased basal and induced AHH activity appears to be a primary characteristic of psoriatic skin; AHH activity might initiate an increase in epidermal cell turnover through modulation of prostaglandin and adenylate cyclase activity.

We have recently suggested that the underlying genetic defect in psoriasis may be related to an abnormality in aryl hydrocarbon hydroxylase (AHH) activity (the cytochrome P450 mixed function oxidase system) since we found that its concentration and capacity for induction are decreased in the clinically normal skin as well as in the lesions of psoriasis (Chapman, Rawlins & Shuster, 1979a). It could be argued that the abnormality in the lesion-free skin was related to a previous involvement by the rash and might therefore be a secondary effect rather than primary. We therefore tested our hypothesis further by looking for the defect in AHH activity in the clinically normal forearm skin of patients with pustular psoriasis which was and always had been localized to the palms and soles. Moreover, since palmo–plantar pustular psoriasis is quite distinct from other forms of psoriasis, the study would further test our hypothesis by demonstrating that the defect was not only confined to one form of the disease.

PATIENTS AND METHODS

Thirteen patients (ten female) agreed to participate in the study. All had unequivocal palmo–plantar

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pustular psoriasis and none had plaque or other forms of psoriasis elsewhere on the body, and the scalp and nails were normal. Only one patient (Patient 13) had a history of discoid psoriasis in the past. Their ages ranged from 35 to 73 years (mean 55±3 s.e. mean). Thirteen healthy volunteers with no history or clinical evidence of skin or other disease were used as controls. Their ages ranged from 18 to 37 years (mean 26±2 s.e. mean).

Epidermal blisters were raised with a suction pump on the flexor aspect of forearm skin both in patients and in healthy volunteers (Kiistala & Mustakallio, 1967). The blister tops, consisting of separated viable epidermis, were removed with sterile forceps and immediately placed in ice-cold isolation medium (20 mmol/l tris-[hydroxymethyl] methylamide in 0·3 mol/l mannitol, pH 7·4). The epidermis was divided into two parts, and each was weighed and transferred to a tissue-culture system (Nebert & Gelboin, 1968), with and without benzanthracene (100 μm). After preincubation for 18 h at 37°C in an atmosphere of 95% O_2:5% CO_2 the activity of AHH in epidermal microsomes was determined as previously described, in the presence of essential cofactors and divalent cations (Chapman, Rawlins & Shuster, 1979b). Benz (α) pyrene was used as substrate, and the activity of AHH was expressed as pmol 3-hydroxybenz(α)pyrene formed per mg microsomal protein/h (pmol 3OH-BP/mg/h). In previous studies (unpublished) we have shown the results are not significantly different when expressed in relation to epidermal DNA.

RESULTS

Basal and induced AHH activities in lesion free epidermis from patients with pustular psoriasis are shown in Table 1. Basal AHH activity (mean 2·32±0·33 s.e. mean pmol 3OH-BP/mg/h) was significantly less (t = 2·73; $P<0·02$) than that of the normal volunteers (mean 3·14±0·23 s.e. mean pmol 3OH-BP/mg/h, Table 2). Preincubation of epidermis from normal volunteers with 100 μm benzanthracene increased the activity of AHH to 6·85±0·79 s.e. mean pmol 3OH-BP/mg/h (see Table 2). By contrast, except in one patient, there was no increase in AHH activity in clinically normal epidermis

TABLE 1. Patients with palmo-plantar pustular psoriasis

			Duration	Involvement		AHH activity (pM 3OH-BP/mg microsomal protein/h)		Induction ratio
Patient	Sex	Age	(years)	Palms	Soles	Basal	Induced	Induced AA activity / Basal AHH activity
1	M	40	17	+	+	2·63	3·14	1·19
2	M	35	0·3	+	0	0·69	1·01	1·46
3	M	71	2	0	+	3·01	3·02	1·00
4	F	62	7	+	0	1·96	1·95	0·99
5	F	53	5	+	+	1·11	2·34	2·11
6	F	49	8	0	+	2·23	2·67	1·20
7	F	51	6	0	+	0·76	0·77	1·01
8	F	40	7	0	+	3·42	2·67	0·78
9	F	61	10	+	0	2·21	2·92	1·32
10	F	63	7	0	+	3·02	2·91	0·96
11	F	57	8	0	+	—	3·06	—
12	F	73	3	+	+	4·70	3·76	0·80
13	F	55	8	+	+	2·09	2·13	1·02
Mean		54·6	6·8			2·32	2·49	1·15
S.e. mean		3·2	1·1			0·22	0·24	0·10

TABLE 2. Normal subjects

Subject	Sex	Age (years)	AHH activity (pM 3OH-BP/mg microsomal protein/h) Basal	Induced	Induction ratio $\frac{\text{Induced AHH activity}}{\text{Basal AHH activity}}$
1	M	30	3·31	6·64	2·01
2	M	37	2·78	5·47	1·97
3	M	18	2·57	4·79	1·86
4	M	30	3·81	8·02	2·10
5	M	28	3·48	6·89	1·98
6	M	30	3·86	4·71	1·22
7	F	22	4·48	8·84	1·97
8	F	25	3·60	5·28	1·47
9	F	21	3·31	7·76	2·34
10	F	30	2·02	4·82	2·39
11	F	37	2·23	2·88	1·29
12	F	21	4·52	14·23	3·15
13	M	24	4·34	8·66	2·00
Mean		27·2	3·41	6·85	1·98
S.e. mean		1·7	0·23	0·79	0·14

from patients with localized pustular psoriasis (Table 1). There was no relationship between basal level of AHH activity and the site of the lesions.

DISCUSSION

The results of this study indicate that the activity of AHH in lesion free epidermis from the forearms of patients with palmo–plantar pustular psoriasis is reduced when compared to normal individuals. There was an even greater difference in the induction of AHH activity by benzanthracene: the activity doubled in skin from normal subjects but with one exception did not change significantly in the clinically normal forearm skin from the patients with pustular psoriasis. The younger age of our control subjects would have minimized the difference, since age and epidermal AHH activity are positively correlated (Chapman *et al.*, 1979b). This abnormality in epidermal microsomal AHH activity is comparable with that previously found in the lesion free skin of patients with the common form of psoriasis (Chapman *et al.*, 1979a). Since all but one of the patients had never had psoriasis, other than on the palms and soles, the present findings show that the defect we found in forearm skin cannot have been secondary to a previous episode of psoriasis. The present findings also show that the abnormality of epidermal microsomal AHH, which we have observed so far only in psoriasis, is not confined to one particular variety of that disease.

It has been postulated that the whole skin is abnormal in psoriasis (Shuster & Marks, 1970) and that the primary defect was likely to be recognized in clinically unaffected skin rather than in the lesions where observed changes might be secondary. A number of abnormalities have been reported in clinically unaffected skin (Gara *et al.*, 1964; Porter & Shuster, 1968; Cooper, McGrath & Shuster, 1976; Greaves *et al.*, 1978) but most are minor in comparison with the impairment of AHH activity and inducibility. The present results therefore support the suggestion that the defect in AHH is closely related to the fundamental abnormality of psoriasis (Chapman *et al.*, 1979a). Thus, either the gene for epidermal AHH and its inducibility is linked to the gene for psoriasis and is thereby closely but not causally related to the disease, or the reduction in AHH activity is causally related to the gene for psoriasis, the reduction itself resulting in the increase in epidermal cell turnover characteristic of the disease. In support of the second possibility is our observation that the increase in epidermal cell turnover induced by removal of the horny layer with cellophane tape is associated with a reduction in AHH activity in the underlying epidermis. Thus an increase in epidermal cell turnover through modulation of prostaglandin production and adenylate cyclase activity may occur secondarily to the reduction in AHH activity.

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Topical spermine and putrescine stimulated DNA synthesis in the hairless mouse epidermis

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SUMMARY

Polyamines were applied topically to the skin of the hairless mouse. Putrescine stimulated the incorporation of thymidine after a 24-h application period. The effect of polyamines upon skin pretreated with a potent topical steroid was also examined; in this model thymidine incorporation was stimulated by both spermine and putrescine. Pretreatment was performed in order to reduce endogenous polyamine biosynthesis and increase the sensitivity of the epidermis to exogenous polyamines. Depletion of the activity of ornithine decarboxylase, the rate-limiting polyamine biosynthetic enzyme, by topical steroids was confirmed in the hairless mouse following induction of the enzyme by UV-B. The results are consistent with those of *in vitro* studies suggesting a role for polyamines in the control of DNA synthesis; the effect of corticosteroids upon proliferative skin disorders may be mediated through this mechanism.

Increased levels of polyamines and their biosynthetic enzymes are closely associated with cell proliferation in many organ systems, including regenerating liver (Janne & Raina, 1968), mitogen induced proliferation of lymphocytes (Fillingame, Jorstad & Morris, 1975), tumour induction (Fujita *et al.*, 1978), and stimulation of fibroblast cultures by epidermal growth factor (DiPasquale, White & McGuire, 1978).

Inhibition of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis, has been shown (Mamont *et al.*, 1976) to block the proliferation of cells in culture; this effect is reversed by the addition of polyamines to the culture medium. In the skin increased levels of ODC have been demonstrated in states of proliferation such as psoriasis (Russell *et al.*, 1978), following UV-B irradiation (Lowe, Verma & Boutwell, 1978), following the application of tumour promoting

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agents (O'Brien, Simsiman & Boutwell, 1975; O'Brien, 1976) and in skin stimulated by hair plucking (Probst & Krebs, 1975; Morrison & Goldsmith, 1978).

Nevertheless, it is not clear whether polyamines *in vivo* are directly implicated in the control of DNA synthesis. In human psoriatic skin, reduction of ODC activity and polyamine levels occurs with steroid treatment (Russell *et al.*, 1978) and is followed by clinical improvement, but a causal relationship has not been demonstrated.

The present study was performed to determine whether polyamines could stimulate epidermal DNA synthesis when applied topically to the skin of the hairless mouse.

DNA synthesis was stimulated by putrescine in normal skin, and by putrescine and spermine in steroid pretreated skin, but no significant increase in DNA synthesis followed application of spermidine. Our findings confirm that polyamines have the potential for stimulating DNA synthesis *in vivo*, which has not previously been demonstrated, and suggest that these compounds could be directly implicated in the mechanism for control of epidermal cell replication *in vivo*.

MATERIALS AND METHODS

Animals

Adult hairless mice were used for all studies.

Polyamine application

Spermine base, spermidine base and putrescine base (Sigma) were dissolved at a concentration of 2·5% in a vehicle of normal saline containing 0·1% Triton-X 100 to aid spreading; 0·02 ml of the polyamine solution or the vehicle alone was applied to the dorsal skin at 24, 23, 22, 21, 20, 19, 3 and 2 h prior to killing.

Corticosteroid pretreated animals received 0·02 ml betamethasone valerate lotion (Valisone) to the dorsal skin 24 h prior to sacrifice. Vehicle alone was applied at this time to the remaining animals.

UV-B irradiation

Mice were irradiated with UV-B provided by two sunlamp bulbs (Westinghouse FS-20, 20 W, output 0·05 mW/cm^2 at 30 cm, measured using an IL500 research radiometer equipped with a UV-B probe) suspended 30 cm above the backs of the mice. Total dose given was 0·05 J/cm^2.

DNA synthesis assay

One hour before killing each animal received 25 μCi ^{3}H TdR (specific activity 1 Ci/mM) in 0·2 ml normal saline, by intraperitoneal injection.

TdR incorporation into the epidermis as a measure of epidermal DNA synthesis was estimated by the method of Otani (1980) which is as follows: the animals were killed by neck dislocation. The dorsal skin was cleansed with ethanol, excised and spread on a paper towel. Two 17 mm transparent semi-rigid plastic discs were fixed to the skin with cyanoacrylate glue; excess glue was blotted from the sample. Skins were then heated for 10 min between metal sheets at 60°C to aid the later separation of dermis. The skin surrounding the discs was cut away, and the discs and adherent skin placed in scintillation vials containing 5 ml of 2 M potassium bromide. The samples were then incubated at 60°C for approximately 1 h or until the dermis had contracted and become detached from the epidermis, which remained adherent to the disc. The dermis and potassium bromide were poured from the vial, and the disc and epidermal sample rinsed and incubated in 0·25% acetic acid either for 1 h at room temperature or overnight at 4°C. Each vial was then rinsed with distilled water followed by 95% ethanol and drained. Then 5 ml Insta-Gel (Packard) was added to the disc. ^{3}H content was determined using a Beckman LS-150 scintillation counter.

Ornithine decarboxylase assay

Enzyme preparation. Animals were killed by neck dislocation; dorsal skin was removed immediately. Epidermis was separated by immersion of the skin in 50 mM phosphate buffer at 56°C for 15 s, followed by immediate cooling in buffer at 0°C; the skin was then spread on paper, epidermis upward. The epidermis was removed by gentle scraping and placed in preweighed tubes surrounded by ice. In most experiments individual skins were assayed; in the remaining experiments epidermis from two to four skins was pooled. The epidermis was homogenized for 15 s in 19 volumes of 50 mM phosphate buffer containing 0·2 mM pyridoxal phosphate, 1 mM EDTA, and 4 mM dithiothreitol, using a Brinkmann homogenizer. The homogenate was centrifuged at 20,000 g for 10 min at 0°C, giving a clear supernatant.

Ornithine substrate. Ornithine hydrochloride (1·2 mM) was dissolved in buffer containing pyridoxal phosphate, EDTA and dithiothreitol as above; 1·0 μCi DL [1-^{14}C] ornithine hydrochloride/0·1 ml substrate was added.

Procedure. Enzyme preparation (0·2 ml) was put in each 15 ml tube; 0·1 ml of labelled substrate was added, and the tube closed with a rubber stopper fitted with a centre well assembly (Kontes) containing a filter paper bearing 0·05 ml NCS tissue solubilizer (Amersham), for CO_2 absorption. The tubes were incubated for 60 min at 37°C in a shaking water bath. The reaction was terminated by the addition of 0·2 ml 2 M citric acid. Incubation was continued for 1 h to ensure complete absorption of $^{14}CO^2$ by the NCS. Filter papers were then transferred to scintillation vials containing 5 ml of Insta-Gel. Each estimation was performed in duplicate; blank tubes contained no enzyme Release of $^{14}CO_2$ was linear up to 1 h.

RESULTS

DNA synthesis in normal skin treated with topical polyamines

Topical putrescine caused a significant increase in DNA synthesis ($P<0{\cdot}005$) (Table I). DNA synthesis was not significantly altered by the topical application of spermine and spermidine over the 22-h period prior to sacrifice.

DNA synthesis was also measured in ventral skin of animals whose dorsal skin was treated with putrescine. No significant rise in DNA synthesis was observed in the distant (ventral) site, suggesting that the effect of putrescine is local, rather than mediated through systemic absorption.

The application of a lower (1%) concentration of putrescine in one experiment did not cause significant elevation of DNA synthesis.

DNA synthesis in steroid-treated skin treated with polyamines

A single application of betamethasone valerate caused a marked depression of DNA synthesis 24-h later (c.p.m. in treated skin 20±10% of mean untreated control value).

Steroid treated animals to which spermine and putrescine were applied during the 24-h period between steroid treatment and killing showed a significant increase in DNA synthesis when compared with steroid treated animals which received only the polyamine vehicle (Table I). Spermidine did not cause a significant increase in DNA synthesis.

ODC synthesis

Activity of ODC in normal mouse skin was not detectable. To evaluate the effect of topical steroid application upon ODC activity in the epidermis, animals stimulated with UV-B 18–24 h previously were employed. ODC activity has been shown to reach a plateau 18 and 40 h after irradiation.

TABLE I. Effect of polyamine application to normal and pretreated skin. Results expressed as c.p.m. ± s.e.mean of ^{3}H TdR, and as a per cent of mean control values ± s.e.mean for pooled experiments

	Normal animals					Topical steroid pretreated animals				
	N	Treated	N	Control	*P*	N	Treated	N	Control	*P*
Putrescine										
Expt. I	5	2465 ± 419	6	1109 ± 174		5	431 ± 101	5	173 ± 12	
Expt. II	5	2990 ± 1337	5	2464 ± 275		5	1087 ± 252	5	446 ± 94	
	5	2229 ± 420	5	1493 ± 767						
Total; % of control mean	15	168 ± 17·5	16	100 ± 9	< 0·005	10	246 ± 38	10	100 ± 10	< 0·005
Spermine										
Expt. I	5	1811 ± 234	4	1547 ± 198		5	350 ± 24	5	231 ± 24	
Expt. II	5	1203 ± 152	5	1082 ± 153		5	491 ± 27	5	285 ± 56	
Total; % of control mean	10	114 ± 10	9	100 ± 9	ns	10	161 ± 8	10	100 ± 10	< 0·001
Spermidine										
Expt. I	5	1592 ± 149	5	1425 ± 86		5	366 ± 85	5	245 ± 18	
Expt. II	5	1254 ± 309	5	1105 ± 178		5	557 ± 221	5	403 ± 37	
Total; % of control mean	10	113 ± 14	10	100 ± 8	ns	10	144 ± 30	10	100 ± 5·5	ns

A single application of steroid caused a significant reduction in ODC activity, to approximately 50% of mean control values, 4–6 h later (treated skin 2·99±0·85 nM CO_2/10 mg tissue/h; control skin 6·07±1·68 nM CO_2/10 mg tissue/h). At 18 h ODC activity was not significantly decreased below control levels.

DISCUSSION

Putrescine stimulated DNA synthesis in normal skin. This has not previously been demonstrated *in vivo*, but is consistent with other studies which have shown a direct correlation between polyamine biosynthetic activity and DNA synthesis in epidermis and hair follicles *in vivo* (Probst & Krebs, 1975; Lowe *et al.*, 1978), and with *in vitro* studies showing that polyamines exert a growth stimulating effect (Janne & Raina, 1968; Fillingame *et al.*, 1975; DiPasquale *et al.*, 1978).

Both putrescine and spermine caused partial reversal of the inhibition of DNA synthesis induced in skin by pretreatment with a potent topical corticosteroid. Steroid pretreatment was performed on the assumption that this would deplete endogenous polyamine levels, thereby rendering the skin more sensitive to topically applied polyamines. Topical steroids have previously been shown to reduce polyamines and ODC activity in psoriatic skin (Russell *et al.*, 1976); we were able to confirm a similar effect upon ODC activity in the hairless mouse, previously stimulated by UV-B irradiation, which induces ODC activity (Lowe *et al.*, 1978).

Our assay was not able to detect the basal ODC activity of normal mouse skin, in accordance with other studies (O'Brien *et al.*, 1975; O'Brien, 1976; Probst & Krebs, 1975). Steroid induced inhibition of ODC activity in the normal animal could not therefore be confirmed with the technique available.

The application of putrescine and spermine to steroid treated animals only partly reversed the inhibition of DNA synthesis induced by prior steroid application, since the degree of stimulation observed following polyamine application was small when compared with the inhibition induced by the steroid. This could be accounted for by inadequate penetration of topical polyamine; furthermore, steroids inhibit other cellular events in addition to polyamine biosynthesis, for which polyamine application would not compensate. In addition, only a limited proportion of the cell population might be susceptible to the effect of spermine or putrescine at the time of application.

Repeated applications of polyamine preparations were required to stimulate DNA synthesis. We believe that an irritant reaction resulting from polyamine application is unlikely, because this would be more likely to be manifest in skin not previously pretreated with steroids. Furthermore, repeated application of a higher (5%) concentration of putrescine caused no visible signs of irritation in normal skin. All control animals received applications of the polyamine vehicle containing Triton-X, to exclude the possibility of a reaction to detergent application.

In psoriasis, increased levels of polyamines (Russell *et al.*, 1978; Proctor *et al.*, 1979) and ODC activity are observed in the entire epidermis, with higher levels in lesional skin. ODC activity is reduced in the lesions by steroid treatment, prior to clinical improvement. Polyamine levels are reduced by treatment with topical steroids (Russell *et al.*, 1978), by the Ingram regimen (Proctor *et al.*, 1979), or by dithranol (Böhlen *et al.*, 1978). Our findings of decreased ODC activity following steroid application to proliferative (UV-B stimulated) epithelium are consistent with these observations and may be an important mechanism in the response of proliferative disorders to steroid treatment. Our study also lends further weight to the concept of a role for polyamines as stimulators of DNA synthesis in states of epidermal proliferation such as psoriasis.

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Protease inhibitor profiles in urticaria and angio-oedema

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SUMMARY

Plasma levels of six protease inhibitors have been measured in patients with chronic urticaria, chronic urticaria with angio-oedema, cold and cholinergic urticaria. In chronic urticaria C1 esterase inhibitor activity was increased compared with a reference control population but there was no detectable abnormality of any other protease inhibitor. Patients with chronic urticaria/angio-oedema showed a reduction in inter-α trypsin inhibitor. They also manifested a rise in C1 esterase inhibitor. In cold urticaria there was a significant lowering of $\alpha 1$ antichymotrypsin. The reduction in $\alpha 1$ antitrypsin in this group probably reflects a genetic difference compared with the control population. Patients with cholinergic urticaria also showed a reduction of $\alpha 1$ antichymotrypsin. The elevated levels of $\alpha 2$ macroglobulin in the three groups are probably due to differences in the mean age of these groups compared with the reference population.

Comparison of levels in subgroups of patients with and without active lesions suggest that a consumptive effect may contribute to the reduced values, although it seems unlikely to account for them entirely. The results suggest that involvement of pharmacologically active products of protein digestion may be involved in the pathogenesis of urticaria and should prompt attempts to identify these agents and encourage trial of medications which lead to inhibition of proteolytic activity in urticaria.

Urticaria and angio-oedema affect up to 15% of the population (Sheldon, Matthews & Lovell, 1954). In some, usually acute forms, a provoking allergen is easily identified, the development of urticaria being presumably an anaphylactic phenomenon. In occasional cases urticaria may be a manifestation of a hypocomplementaemic vasculitis (Soter, Austen & Gigli, 1974; Mathison *et al.*, 1977). Local release of histamine can be readily demonstrated in some forms of urticaria (Greaves & Sondergaard, 1970; Bentley-Phillips, Black & Greaves, 1976) but its relevance to the pathogenesis of urticaria remains uncertain (Bentley-Phillips, Eady & Greaves, 1978). Recent attention has been focused on the possible role of tissue or plasma biochemical abnormalities in the pathogenesis of urticaria and angio-oedema.

That the rare hereditary angio-oedema is associated with a deficiency of inhibitor of the first com-

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ponent of complement (C1-esterase) has long been recognized (Donaldson & Evans, 1963). The possibility that reduction of plasma protease inhibitors might be a feature of other forms of urticaria/angio-oedema has been raised by the observation that intradermal injection of the kinin-forming esterase kallikrien causes an amplified wealing reaction in chronic urticaria (Juhlin & Michaëlsson, 1969) and the report by Doeglas & Bluemink (1974) that patients with chronic urticaria had significantly reduced plasma levels of α1-antitrypsin and antichymotrypsin. Since the products of plasma esterase activation might have a role in the pathogenesis of urticaria, the plasma levels of a series of protease inhibitors were determined in patients with several types of chronic urticaria and angio-oedema.

PATIENTS AND METHODS

All patients studied had been referred to St John's Hospital for Diseases of the Skin for investigation and treatment of urticaria. Patients with chronic urticaria manifested extensive wealing occurring daily or almost daily for at least 3 months. Chronic urticaria/angio-oedema patients were defined as above but in addition suffered recurrent extensive swellings of subcutaneous tissues. Patients with cold urticaria showed a reproducible positive wealing reaction to contact with ice and had suffered cold sensitivity for at least 3 months. Cholinergic urticaria was diagnosed by recently published clinical criteria (Commens & Greaves, 1978). The following investigations were carried out on all patients: full blood count, ESR, antinuclear factor (ANF), blood urea and electrolytes, liver function tests, total plasma proteins, immunoglobulins IgG, IgA, IgM and IgE, serum cryoglobulin, cryofibrinogen, cold agglutinins, WR and stool for ova and parasites. Total plasma C3 and C4 were also estimated. All treatment was withdrawn for at least 2 days prior to study.

For protease inhibitor studies 10 ml venous blood was obtained, of which 5 ml was allowed to clot at ambient temperature and a second 5 ml was anticoagulated with potassium EDTA (ethylene diaminetetracetic acid).

Protease inhibitors

Serum levels of α1-antitrypsin (α1AT) were estimated by automated immunoprecipitation and of α1-antichymotrypsin (ACT), inter-α-trypsin inhibitor (IαTI) and α2 macroglobulin (α2M) by single radial immunodiffusion. Plasma levels of antithrombin III (AT/III) and C1 esterase inhibitor (C1 INH) were estimated by single radial immunodiffusion. Monospecific antisera were obtained from Atlantic Antibodies, Inc., Westbrook, Maine (α1AT and C1 INH) and Behringwerke Ag, Marburg/Lehn. Mass concentration of the proteins was expressed in relation to the Supra Regional Protein Reference Unit Standard (α1AT) and standard preparations obtained from Behringwerke AG. α1-Antitrypsin phenotypes were determined by isoelectric focusing in polyacrylamide gels and by extended agarose electrophoresis at pH 8·6 (Milford Ward *et al.*, 1977).

RESULTS

The antitrypsin (Pi) phenotypes of the patients studied are shown in Table 1. No group differs significantly from the reference population save possibly for the modest increase in Pi MZ in the cold urticaria group. The total number of cases in this group is, however, too small to attach any significance to this observation. The protease inhibitor levels in the four groups are summarized in Table 2.

Chronic urticaria

Forty-two patients aged 13–64 years (twenty males) were studied, thirteen of whom had a history sug-

TABLE 1. Pi phenotypes in urticaria

	n		M	MS	MZ	Other
Chronic	42	o	38	2	2	—
		e	34	4	1	3
Chronic with angio-oedema	15	o	13	—	1	1
		e	12	1	<1	1
Cholinergic	26	o	25	—	1	—
		e	21	2	<1	2
Cold	18	o	14	—	4	—
		e	15	2	<1	1

gestive of atopy. All had suffered multiple weals daily for at least 3 months and nine had symptoms of arthralgia. Twenty-three had active lesions at the time of study. Of the six protease inhibitors measured, only C1 inhibitor (0·41±0·08 g/l; mean±s.d.) differed significantly from the control population (0·33±0·06) g/l) ($P<0{\cdot}001$). Plasma C3 and C4 values were normal.

Cholinergic urticaria

Thirty-eight samples were obtained from twenty-six patients aged 15–42 years (fifteen males), eight of which had a history of atopy. Fifteen samples were collected from patients with active lesions. Of the six protease inhibitors, only α1-antichymotrypsin (0·44±0·07 g/l) showed a significant variation from the control group (0·56±0·15 g/l) ($P<0{\cdot}001$). Although α2 macroglobulin values were increased compared with the control, this difference became non-significant with age correction of the control population. Plasma C3 and C4 values were within normal limits.

Cold urticaria

Nineteen samples were obtained from eighteen patients aged 11–52 years (seven males), three of whom had a history suggestive of atopy. Three patients had symptoms of arthralgia and four had active lesions at the time of study. α1-Antichymotrypsin (0·47±0·11 g/l) showed a significant reduction compared with the control population (0·56±0·15 g/l) ($P<0{\cdot}05$). The reduced level of α1-antitrypsin for the total group is related to the modest increase in the Pi MZ phenotype. α2-Macroglobulin values are not significantly different from an age-corrected reference population. Plasma C3 and C4 values were within normal limits.

Chronic urticaria with angio-oedema

Seventeen samples were collected from fifteen patients, aged 25–51 years (four males), of whom three had a history of atopy. Six patients had active lesions at the time of study. C1 inhibitor levels (0·40±0·05 g/l) were increased compared with control (0·33±0·06 g/l) ($P<0{\cdot}001$), and inter-α-trypsin inhibitor levels (0·48±0·07 g/l) were reduced compared with control (0·57±0·12 g/l) ($P<0{\cdot}01$). Values for the other protease inhibitors and for plasma C3 and C4 were within normal limits.

Activity of urticaria

To assess the influence of activity of urticaria on the level of protease inhibitors, values in patients with active lesions were compared with those from patients who were clinically inactive at the time of study. Further samples were obtained from patients before and after specific challenge designed to

TABLE 2. Protease inhibitor levels in urticaria (mean ± s.d.) (g/l)

	n	α1AT	α2M	ACT	IαTI	AT III	C1 INH
Chronic	42	2·01±0·35	3·01±0·99	0·55±0·15	0·55±0·10	0·34±0·04	0·41±0·08 *P*< 0·001
Chronic with angio-oedema	17	2·09±0·41	3·65±1·51 *P*< 0·05	0·58±0·13	0·48±0·07 *P*< 0·01	0·32±0·04	0·40±0·05 *P*< 0·001
Cholinergic	38	1·96±0·33	3·74±0·82 *P*< 0·01	0·44±0·07 *P*< 0·001	0·53±0·08	0·32±0·06	0·35±0·09
Cold	19	1·83±0·27 *P*< 0·001	3·75±1·03 *P*< 0·01	0·47±0·11 *P*< 0·05	0·59±0·14	0·33±0·05	0·35±0·05
Normal population		2·10±0·30	3·00±0·90	0·56±0·15	0·57±0·12	0·34±0·05	0·33±0·06

TABLE 3. Protease inhibitor levels in relation to disease activity (mean ± s.d.) (g/l)

		n	α1AT	α2M	ACT	IαTI	AT III	C1 INH
Chronic	Active	23	2·08±0·31	2·93±1·00	0·56±0·13	0·55±0·10	0·34±0·04	0·42±0·07
	Inactive	19	1·93±0·38	3·09±0·99	0·54±0·18	0·55±0·09	0·33±0·05	0·41±0·09
Chronic with angio-oedema	Active	6	2·13±0·50	3·15±1·48	0·53±0·12	0·46±0·04	0·30±0·04	0·40±0·07
	Inactive	11	2·06±0·39	3·92±1·53	0·61±0·13	0·49±0·08	0·34±0·04	0·40±0·05
Cholinergic	Active	15	1·99±0·34	3·73±0·78	0·43±0·08	0·54±0·09	0·35±0·07	0·37±0·09
	Inactive	23	1·94±0·32	3·75±0·87	0·45±0·07	0·51±0·07	0·31±0·05	0·37±0·07
Cold	Active	4	1·83±0·15	3·88±1·61	0·41±0·09	0·69±0·10	0·32±0·06	0·35±0·06
	Inactive	15	1·83±0·30	3·71±0·89	0·48±0·11	0·57±0·14	0·33±0·05	0·36±0·05

produce urticarial weals. The results are summarized in Table 3. Whilst differences between the mean protease inhibitor levels in active and inactive disease did not approach significance with these small number groups, certain trends are evident. The reduction of α1-antichymotrypsin seen in the total cholinergic and cold urticaria groups is further exaggerated in active disease. In chronic urticaria with angio-oedema, both inter-α-trypsin inhibitor and antithrombin III show lower values in active than inactive disease. In contrast, the elevated C1 inhibitor levels in both forms of chronic urticaria are not affected by disease activity, and the phenotypic reduction of α1-antitrypsin in cold urticaria is similarly unaffected by activity.

Other laboratory investigations

Of forty-two patients with chronic urticaria three had an ESR of 21–44 mm in the first hour. One had an ANF of 1:40, and one a rheumatoid factor of 1:64. Liver function tests were abnormal in five patients (gamma glutamyl transpeptidase and alkaline phosphatase raised in one, alkaline phosphatase, lactate dehydrogenase and total bilirubin raised in one; alanine transaminase raised in one; alkaline phosphatase raised in one and serum bilirubin raised in one). One patient had a raised serum albumin and reduced serum globulin, and one patient had a positive WR. One patient had evidence of intestinal parasitic infestation. Immunoglobulin analysis revealed a raised IgA and reduced IgM in one patient, and a lowered IgA in a second patient. Raised serum IgE levels (570–1750 units) were found in four. Of the fifteen patients with chronic angio-oedema urticaria, one had an ANF of 1:32; and immunoglobulin analysis revealed a raised IgM. Of the twenty-six patients with cholinergic urticaria, one patient had a raised serum bilirubin and a low serum iron. Two patients had a raised IgM, one patient had a raised IgA and one had a low IgA. Raised serum IgE level (1050 iu) was found in one patient. Of the eighteen patients with cold urticaria, one had a low serum potassium. A raised IgM was found in one patient (750 iu). Four patients had a cold haemolysin titre at 1/16, and one patient a titre of 1/32.

DISCUSSION

The protease inhibitors are a family of plasma proteins which share a common function—that of proteolytic enzyme inhibition (Laurell, 1975). Most of the members of the family exhibit a broad spectrum of activity, being capable of inhibition of many different, although often related, proteolytic enzymes. Whilst most are acute phase reactants, their concentrations rising in response to tissue injury and inflammation, some are 'suicidal' in that the 1:1 molar complex of protease and inhibitor is removed from the circulation by the reticuloendothelial system.

Whilst α1-antitrypsin is responsible for 75% of the plasma tryptic inhibitory capacity in molar terms, the differences in molecular size between various members of the family affect their relative tissue distribution and hence their functional relevance at the tissue level. The relationship between protease inhibitors and the pathogenesis of various forms of urticaria may lie in their relative ability to inhibit kinins and kinin-like substances at the local, or tissue, level. This need not, necessarily, be reflected in gross differences in circulating plasma levels, and will be intimately associated, in the acute episode, with the kinetics of protein synthesis and catabolism. More fundamental aspects of specific protein synthesis, including genetic control and phenotypic representations of individual variation, may also be of relevance.

The central role of C1 esterase inhibitor deficiency in the pathogenesis of the lesions in hereditary angio-oedema has long been recognized. Our results raise the possibility that reduced protease inhibitor levels may also play a part in the pathogenesis of oedema in other chronic forms of urticaria or angio-oedema. A significant lowering of α1-antichymotrypsin was found in eighteen patients with acquired cold urticaria. This finding is of especial interest in view of the recent direct evidence that cold induced urticaria is independent of histamine release (Bentley Phillips *et al.*, 1978), and suggests that other non-histamine mediators, possibly peptide in nature, may be involved in the wealing of cold urticaria. This finding, together with the reductions in $\alpha 1$-antitrypsin, is in agreement with the findings of Doeglas & Bleumink (1975). The apparent reduction in $\alpha 1$-antitrypsin in this series is a reflection of the modest increase in Pi MZ frequency in the group and may be a chance finding rather than a true reflection of a pathogenetic factor. Patients with cholinergic urticaria also showed a significant reduction in $\alpha 1$-antichymotrypsin. This inhibitor is the most specific member of the family, having apparent inhibitory activity against chymotrypsin and leukocyte protease only, and it is noteworthy that cholinergic urticaria is almost completely unresponsive to anti-histamine treatment. The finding of an elevated $\alpha 2$-macroglobulin in three of the groups is not of pathogenetic significance and is related to the age dependent values for this protein. Compared with the cold and cholinergic urticaria groups, patient with chronic urticaria exhibiting angio-oedema show a specific protease inhibitor profile, inter trypsin-α-inhibitor being reduced and C1 inhibitor increased. The reduction in inter-α-trypsin inhibitor may explain the recent successful treatment of patients in this group with a synthetic esterase inhibitor (Thompson & Felix Davies, 1978). The fourth and largest group, patients with chronic urticaria, showed only an increase in C1 inhibitor without difference between active and inactive disease.

Although analytical differences between active and inactive disease in the cold, cholinergic, and angio-oedema urticaria groups did not reach significant levels, the tendency towards lower levels of the inhibitors in active disease suggests that at least part of the reduction is a consumptive phenomenon although consumption is unlikely to account for the reduced levels entirely. Thus our results point to involvement of a pharmacologically active component in the pathogenesis of the urticaria.

Taken together, our results suggest that in some forms of chronic urticaria increased proteolytic activity may play an important role in the pathogenesis of urtication. Direct measurement of esterases and peptide products of protein digestion including kinins in body fluids of patients with urticaria would clearly be of great interest. Our results should also encourage the clinical evaluation of anti-esterase drugs in cold, cholinergic, and angio-oedema urticaria. The possibility that anabolic steroids which enhance protease inhibitor activity might be of value in these conditions deserves exploration since this class of agent is known to be very effective in the prevention of symptoms in the $C\bar{1}$ inhibitor deficiency syndrome, hereditary angio-oedema (Gelfand *et al.*, 1976).

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Studies on the blood fibrinolytic enzyme system of patients with cutaneous vasculitis

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SUMMARY

The fibrinolytic enzyme system in thirty-two patients suffering from cutaneous vasculitis was investigated. In five of seven patients with Behçet's disease and in five of thirteen patients with erythema nodosum a significant elongation of euglobulin lysis time was observed. On the other hand, in two patients with allergic vasculitis, a significant shortening of euglobulin lysis time was noticed. In the present study, factors responsible for the change of euglobulin lysis time were sought. It was confirmed that the significant elongation of euglobulin lysis time in patients with erythema nodosum was based on an increase of fibrinogen content in the euglobulin fraction. On the other hand, it was speculated that the significant elongation of euglobulin lysis time in patients with Behçet's disease might be due to an increase of antiactivators in the patients' plasma. In the present study, the authors further investigated the status of this antiactivator which is increased in plasma of patients with Behçet's disease, and proposed the possibility that the increased antiactivator might consist of at least two types of antiactivators, one of which is considered to be α2-plasmin inhibitor.

Many reports have accumulated on the role of the fibrinolytic enzyme system in cutaneous vasculitis. Some authors (Cunliffe, 1968; Isacson *et al.*, 1970; Cunliffe & Menon, 1971) have demonstrated that some patients with cutaneous vasculitis have significantly reduced fibrinolytic activity. On the other hand, other authors (Vaithiainathan & Takats, 1968; Kurban & Ryan, 1969) have reported that there is no difference in the blood fibrinolytic activity. In 1973, Chaek, Arnowski & Izak, indicated that the decreased fibrinolytic activity in Behçet's disease might be due to the presence of an inhibitor of plasminogen activator (antiactivator). Sun *et al.* (1976) also reported that the diminished tissue lysis in the skin of patients with cutaneous vasculitis might be due to a decrease of a plasminogen activator which is supposed to be localized in the vascular wall. Although Cunliffe & Menon (1971) reported decreased blood fibrinolytic activity in six of nine patients with erythema nodosum and Chaek *et al.* (1973) also in six of nine patients with Behçet's disease, it is still unclear what factors are responsible for the decreased activity. It is speculated that the change of euglobulin lysis time (ELT) may

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be induced by changes in the fibrinogen, plasminogen and free plasminogen activator contents in the euglobulin fraction. Therefore, in the present study, to elucidate the factors involved in changes of ELT, the fibrinogen, plasminogen and antiactivator contents were determined.

PATIENTS AND METHODS

Human plasminogen and standard plasmin were prepared according to the method described in previous reports (Toki, Ishihara & Yamura, 1977; Toki *et al.*, 1978).

Patients

Thirty-two patients with a vasculitis predominantly involving the skin were studied and the results of the determination were compared with those of the blood fibrinolytic activity of a control group studied in previous reports (Ishihara, Toki & Yamura, 1974; Toki, Hayashi & Takasugi, 1979). These patients were not receiving any medication at the time of the investigation. Seven of the thirty-two patients were diagnosed as having Behçet's disease according to the accepted criteria of Curth (1946). Thirteen patients had erythema nodosum and five had recurrent aphthosis. Three were patients with conditions such as Majocchi-Schamberg disease. Two patients had purpura and skin ulcers and were diagnosed as allergic vasculitis according to Ruiter's criteria (Ruiter & Brandsma, 1948). Two patients were diagnosed clinically as having thrombophlebitis.

Preparation of plasma

Venous blood samples were drawn from each patient before breakfast. One volume of 3·8% sodium citrate was added to nine volumes of blood sample, and plasma was obtained by centrifugation of the mixture at 1580 g for 10 min at 4°C. Plastic equipment and previously siliconized glassware and syringe needles were used to prevent activation of coagulation or fibrinolytic factors.

Measurement of plasma ELT

The measurement of ELT was performed by the method of Sherry *et al.* (1959). The euglobulin fraction was precipitated from 0·5 ml of plasma according to the method of Milstone (1972) (19 volumes of water containing 1·32 volumes of 1% acetic acid added to 1 volume of plasma). After centrifugation for 15 min at 1580 g, the precipitate was dissolved with 0·5 ml of 0·01 M Veronal buffer (pH 7·4 containing 0·1 M NaCl), and immediately clotted with 0·5 ml of thrombin solution (50 units/ml). Clots were incubated at 37°C and the time for complete lysis was recorded by a Clot Lysis Time Recorder (Riko Shoji Co., Ltd).

Measurement of fibrinogen content in euglobulin fraction

The fibrin clots of the euglobulin fraction were prepared by the method described above. The clots were washed completely by saline solution and dried at room temperature. Then the dried clots were hydrolysed with 0·1 ml of 10% sodium hydroxide in a boiling bath for 30 min. The amount of hydrolysed protein was measured by the method of Lowry, Farr & Randall (1951).

Measurement of plasminogen content in euglobulin fraction

The euglobulin fraction was separated from human plasma by the method described above and dissolved with the original plasma volume of 0·1 M phosphate buffer at pH 7·4. To determine the plasminogen content, 0·1 ml of Urokinase (UK) (Green Cross Co., Ltd) solution (1000 iu/ml 0·1 M phosphate buffer at pH 7·4) was added to test tubes containing 0·1 ml of euglobulin fraction, 0·25 ml of 8% casein (Merck) and 0·55 ml of 0·1 M phosphate buffer at pH 7·4. After incubation at 37°C for

1 h, the reaction was stopped by the addition of 10% perchloric acid and plasminogen content was measured by Lowry's method (1951) and expressed in casein units (CU).

L-lysine sepharose affinity chromatography

Two types of antiplasmins and antiactivator contents in plasma were measured by L-lysine sepharose affinity chromatography according to our previous reports (Ishihara *et al.*, 1974; Toki *et al.*, 1979). One and a half millilitres of plasma sample was chromatographed on a L-lysine sepharose column (Daiichi Pure Chem. Co., Ltd) (1·2 × 1·0 cm) equilibrated with 0·1 M phosphate buffer at pH 7·4. After the column was eluted with 4·5 ml of the same buffer, it was washed with 30 ml of the same buffer, and then eluted with 6 ml of 0·1 M acetic acid. As described in previous reports, it was confirmed that the non-adsorbed fraction (6 ml) contained two types of antiplasmins and antiactivators, while the adsorbed fraction (6 ml) contained plasminogen.

Measurement of two types of antiplasmin contents

To determine the immediate type of antiplasmin content, 0·45 ml of standard plasmin (containing 1·5 CU) was added to test tubes containing 0·3 ml of the non-adsorbed fraction and 0·25 ml of 8% casein. After incubation at 37°C for 30 min, the caseinolytic reaction in the tubes was stopped. The immediate type of antiplasmin content was calculated from the difference of caseinolytic activity between the absence and presence of non-adsorbed fraction in the reaction mixture. To determine the slow type of antiplasmin content, 1·5 CU of standard plasmin was incubated with 0·3 ml of the non-adsorbed fraction at 25°C for 30 min, to this mixture was added 0·25 ml of 8% casein, and these were reincubated at 37°C for 30 min. Total antiplasmin content was calculated as immediate type, and the slow type of antiplasmin content was expressed as the difference in units between total antiplasmin and immediate type of antiplasmin. One unit of antiplasmin was defined as the amount of inhibitor capable of completely inhibiting 1 CU of standard plasmin.

Measurement of antiactivator content

To determine the antiactivator content in non-adsorbed fraction, 40 Christensen units (ChU) of streptokinase (SK) (Lederle Lab.) or 40 international units (iu) of UK was added to test tubes containing 1 CU of plasminogen, 0·3 ml of non-adsorbed fraction and 10 μmol of α-N-ρ-Tosyl-L-lysine methyl ester (TLMe) (Nakarai Chem. Co., Ltd). After incubation for 1 h at 37°C, plasmin activity was measured by Hestrin's method (Hestrin, 1949). The antiactivator content was calculated from the difference of plasmin activity between test tube and control tube which does not contain the non-adsorbed fraction. In the present study, 1 unit of antiactivator was defined as the amount of inhibitor which inhibits completely 1 unit of SK or UK.

Measurement of Antistreptokinase antibody (ASK) and Antistreptolysin-O (ASLO) titre

ASK titre was determined by Latex agglutination test according to Kumagai, Ishii & Kobayashi (1968), and ASLO titre was measured by the dilution method of Rantz & Randall (1945).

Gel filtration

A 2·4 × 60 cm column of Sephadex G-200 (Pharmacia) was equilibrated with 0·1 M phosphate buffer at pH 7·4. Ten millilitres of the sample were applied to the column and eluted with the same buffer. The flow rate was 18 ml/h, and 5 ml fractions were collected.

RESULTS

Measurement of ELT

As shown in Table 1, a significant elongation of ELT was noticed in five of seven patients with

TABLE 1. Measurement of fibrinolytic enzyme systems in patients with cutaneous vasculitis

Case no.	Diagnosis	Age (years)	Sex	ELT (min)	Fibrinogen (mg/dl)	Plasminogen (CU/ml)	Antiplasmin (u/ml)		Antiactivator (u/ml)	
							Immediate	Slow	Anti-SK	Anti-UK
1	Behçet's disease	35	M	960	330	6·01	5·55	6·05	530	490
2		29	M	850	350	5·98	5·78	5·95	490	480
3		42	F	1040	320	5·75	5·63	5·87	660	480
4		19	M	360	300	5·88	5·40	5·96	270	410
5		28	F	1120	250	5·46	5·85	5·75	620	470
6		33	M	410	310	4·89	5·73	5·42	290	390
7		41	M	780	300	5·23	5·60	5·56	480	460
8	Erythema nodosum	43	F	760	430	5·42	5·45	5·39	310	370
9		38	F	420	240	5·65	5·58	5·80	290	380
10		35	F	680	380	6·01	5·67	5·85	280	350
11		27	M	390	210	5·84	5·55	5·75	260	410
12		16	M	350	180	5·76	5·90	6·01	290	400
13		23	F	890	190	6·25	5·85	6·20	270	380
14		39	F	370	230	5·98	5·45	5·97	260	360
15		48	F	1110	450	5·57	5·73	5·65	270	370
16		53	F	330	310	5·93	5·82	5·44	300	410
17		34	M	405	270	5·55	5·65	5·48	290	400
18		35	F	400	230	4·87	5·54	5·59	250	350
19		19	M	540	390	6·10	5·57	5·73	270	390
20		21	F	380	240	5·55	5·97	5·38	260	370
21	Recurrent aphthosis	17	F	410	170	4·88	5·63	5·44	270	350
22		31	F	360	180	5·39	5·58	5·63	280	370
23		34	M	370	230	5·25	5·71	5·90	260	380
24		43	F	340	310	6·01	5·59	6·12	290	400
25		28	M	420	220	5·93	5·80	6·05	270	360
26	M–S disease*	27	M	380	275	5·53	5·70	5·56	270	350
27		56	M	405	310	5·47	5·75	5·87	280	340
28		29	M	350	230	5·24	5·78	5·99	270	360
29	Allergic vasculitis	48	M	280	210	4·98	4·91	6·13	270	370
30		65	F	240	185	2·70	5·12	5·80	280	410
31	Thrombophlebitis	37	M	390	200	4·70	5·72	5·78	290	380
32		39	M	380	240	4·95	5·70	5·50	280	410
Physiological range (mean ± s.e.)				375 ± 50	250 ± 100	5·4 ± 0·8	5·7 ± 0·3	5·75 ± 0·45	278·5 ± 27·3	338·5 ± 53·2

Underlines show the abnormal titres.

* Majocchi–Schamberg disease.

Behçet's disease and in five of thirteen patients with erythema nodosum, while a significant shortening of ELT was noticed in two patients with allergic vasculitis. No significant change of ELT was noticed in any of the patients in the other groups.

Measurement of fibrinogen content in euglobulin fraction

As shown in Table 1, in Behçet's disease group the fibrinogen content in the euglobulin fraction remained within the physiological range in spite of a significant elongation of ELT. Contrarily, in four of five patients with erythema nodosum, a significant increase of the fibrinogen content was recognized. On the other hand, the two patients with allergic vasculitis showed a physiological fibrinogen content in spite of a significant shortening of ELT. In all other patients without significant change of ELT, the fibrinogen content also remained within the physiological range.

Measurement of the plasminogen content in euglobulin fraction

As shown in Table 1, in all patients except one of two patients with allergic vasculitis no significant change of plasminogen content in the euglobulin fraction was recognized.

Measurement of two types of antiplasmin content and antiactivator

As shown in Table 1, although a slight decrease in the immediate type of antiplasmin content which is supposed to be $\alpha 2$-macroglobulin (Norman, 1958) was recognized in two patients with allergic vasculitis, all other patients showed no significant immediate type of antiplasmin content. On the other hand, the slow type of antiplasmin content which is supposed to be $\alpha 1$-antitrypsin (Norman, 1958) remained within the physiological range in all patients. As shown in Table 1, the five patients in the Behçet's disease group who showed significant elongation of ELT demonstrated significant increase of both anti-SK and anti-UK contents in plasma. It was of particular interest that the increase of anti-SK content was more than the increase of anti-UK content, whereas the anti-UK unit was larger than the anti-SK unit in the physiological range. On the other hand, all except these five patients failed to show a significant antiactivator content in plasma.

Measurement of ASK and ASLO titre in patients' serum

As shown in Table 2, although there was a significantly high titre of ASK in one of seven patients and a significantly high titre of ASLO in two patients no correlation was noticed between anti-SK and the ASK titres.

TABLE 2. Measurement of ASK and ASLO in patients with Behçet's disease

Case no.	Anti-SK	ASK	ASLO (Todd units)
1	530	×320	166
2	490	×320	100
3	660	×640	100
4	270	×1280	333
5	620	×320	166
6	290	×5120	333
7	480	×320	100
Normal range		⩽×1280	⩽166

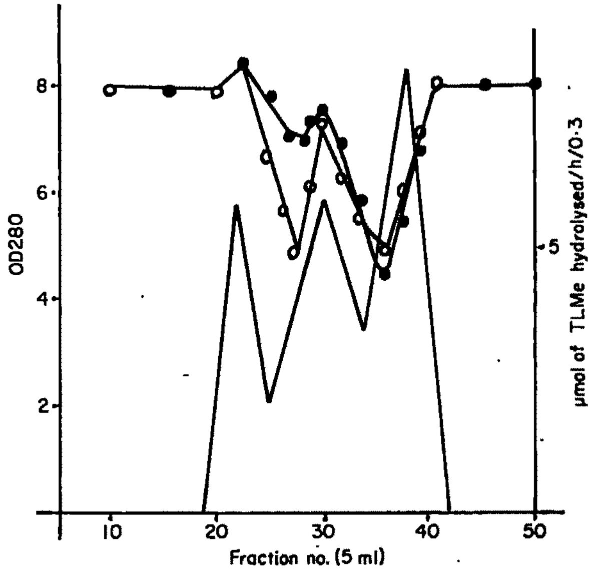

FIGURE 1. Gel filtration of patients' plasma on Sephadex G-200 and antiactivator activity of each fraction. Ten millilitres of plasma containing 620 units of anti-SK and 470 units of anti-UK was chromatographed on Sephadex G-200. To determine the antiactivator activity in each fraction, 100 ChU of SK or 100 iu of UK was added to test tubes containing 1 CU of plasminogen, 0·1 ml of each fraction and 10 µmol of TLMe. After incubation for 1 h at 37°C, plasmin activity was measured by Hestrin's method. (——) Protein; (o——o) anti-SK; (●——●) anti-UK.

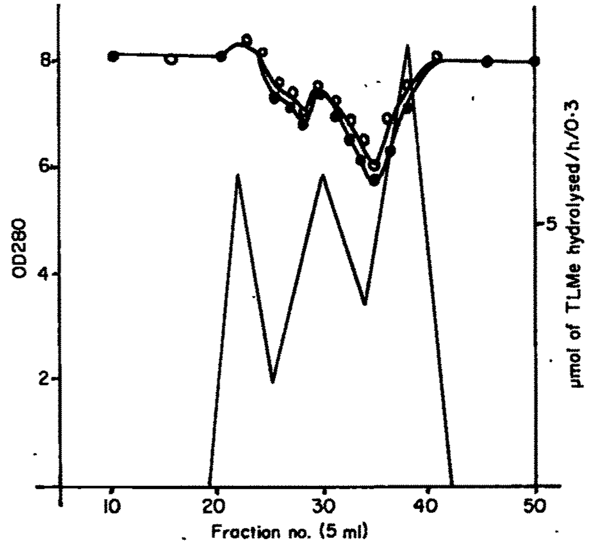

FIGURE 2. Gel filtration of normal plasma on Sephadex G-200 and antiactivator activity of each fraction. Ten millilitres of plasma containing 285 units of anti-SK and 365 units of anti-UK was chromatographed on Sephadex G-200. The antiactivator activity was determined by the method described in Fig. 1. (——) Protein; (o——o) anti-SK; (●——●) anti-UK.

Fractionation of antiactivators in plasma of patients with Behçet's disease

The distribution of proteins, anti-SK and anti-UK activities are shown in Fig. 1. Protein was eluted as three main peaks and both anti-SK and anti-UK activities were eluted in fractions immediately preceding the γ-globulin fractions and albumin fractions. The anti-SK activity in the first antiactivator fraction was remarkably higher than the anti-UK activity, while in the second antiactivator fraction the anti-SK activity was slightly lower than the anti-UK activity. From these findings it was confirmed that the difference between anti-SK and anti-UK units in patient plasma might be due to the difference between anti-SK activity and anti-UK activity in the first antiactivator fraction.

Fractionation of antiactivators in plasma of normal persons

As shown in Fig. 2, smaller amounts of anti-SK and anti-UK activities than those in patients' plasma were eluted in the fractions immediately preceding the albumin fractions, and almost the same amounts of anti-SK and anti-UK activities as anti-UK activity in the first fraction of patient plasma were detected in fractions immediately preceding the γ-globulin fractions.

DISCUSSION

As shown in Table 1, in four of five patients with erythema nodosum who had significant prolongation of ELT a significant increase of fibrinogen content in the euglobulin fraction was confirmed, whereas the plasminogen content remained within the physiological range. This result makes us strongly suspect that the significant increase of fibrinogen content may be one of the important factors which induces decreased fibrinolytic activity in erythema nodosum. On the other hand, in all patients with Behçet's disease there was no significant increase in the content of fibrinogen or plasminogen in the euglobulin fraction, which was contradictory to the results of Chaek *et al.* (1973) who had reported a marked elevation in plasma fibrinogen content and a pronounced decrease of fibrinolytic activity. The contradictory results may be based on the difference in the determination method or difference between the plasma and euglobulin fractions. What then is responsible for the significant prolongation of ELT in this study? Chaek *et al.* (1973), on the basis of the determination of antiplasmin and antiactivator levels by the fibrin clot method using ^{125}I-fibrinogen, proposed that the decreased activity might have resulted from the presence of potent antiplasmin activity and antiactivator activity in patients' sera. Therefore, in the present study, two type of antiplasmins which were confirmed to be α2-macroglobulin and α1-antitrypsin (Norman, 1958) and antiactivator contents in plasma of patients with cutaneous vasculitis were measured respectively by L-lysine sepharose affinity chromatography. As shown in Table 1, contrary to the results by Chaek *et al.* (1973) the two types of antiplasmin in plasma of patients with Behçet's disease were within the physiological range. On the other hand, in the determination of antiactivator using SK or UK, significant increases of both anti-SK and anti-UK contents were recognized in five of seven patients with Behçet's disease who had significant elongation of ELT. From this result, it was suggested that the decrease of activator content in the euglobulin fraction which is followed by an increase of antiactivator content in plasma might induce the significant prolongation of ELT.

It has been proposed that the free plasminogen activator might be excreted from epithelial cells into blood and circulate in the free form, and that spontaneous blood fibrinolytic activity as shown in ELT might reflect free activator activity (Astedt, Bergentz & Svanberg, 1974; Donner, Klener & Roth, 1977; Johnson & Mansfield, 1978). However, the status of activator has yet to be clarified. Moreover, the status of antiactivator is also still unclear. In a preliminary report (Toki *et al.*, 1979) the authors confirmed the existence of antiactivators in normal human plasma and established a new assay method for antiactivators. In the present study the significance of antiactivator, which is increased in plasma of patients with Behçet's disease, was investigated. From the results of determination of antiactivator, it was of particular interest that while anti-UK units in patients' plasma were slightly higher than the physiological range, anti-SK units were remarkably higher. These results lead us to speculate that the chief component of the antiactivator which is increased in patients' plasma may be an inhibitor which inhibits SK more effectively than UK. The relationship between Behçet's disease and ASK has been discussed by many workers. Fletcher, Alkjaersig & Sherny (1959) have indicated that, as a result of previous streptococcal infection, antibodies which cross-react with and neutralize SK are produced. Kaneko *et al.* (1972) reported that significantly high titres of ASK were recognized in 70% of patients with Behçet's disease. The authors first assumed that the entity might be ASK; however, as shown in Table 2, no correlation was confirmed between ASK titre and anti-SK.

To characterize the antiactivator, patient plasma was chromatographed on Sephadex G-200. As shown in Fig. 1, protein was eluted as three main peaks and both anti-SK and anti-UK activities were eluted in fractions immediately preceding the γ-globulin and albumin fractions. While the anti-SK activity in the first antiactivator fraction was remarkably higher than the anti-UK activity, the anti-SK activity in the second antiactivator fraction was slightly lower than the anti-UK activity. To compare the antiactivators in patients' plasma with those in normal plasma, normal plasma was also chromatographed on Sephadex G-200. As shown in Fig. 2, although the antiactivator activity in the second antiactivator fraction of normal plasma was lower than that of patients' plasma, both the anti-SK and anti-UK column patterns of normal plasma were almost the same as the anti-UK column pattern of patients' plasma. From comparison of the column pattern between patients' and normal plasma, the following possibilities were speculated: (1) at least two types of antiactivators are present in plasma of healthy persons as well as in that of patients with Behçet's disease, (2) the higher anti-UK units in plasma of patients with Behçet's disease than in normal plasma is due to the difference of anti-UK activity in the second antiactivator fraction between patients' and normal plasma; and (3) the remarkably higher anti-SK units than the anti-UK units in plasma of patients with Behçet's disease is also due to the difference between anti-SK and anti-UK activities in the first antiactivator fraction.

In 1961 Nilsson *et al.* reported that an inhibitor which can inhibit the activation of plasminogen by SK or UK was present in human plasma α2-globulin fraction and in 1970 Hedner, Nilsson & Jacobsen reported that this inhibitor could be eluted in the fractions immediately preceding albumin fractions on Sephadex G-200 column chromatography. In 1977 Aoki *et al.* confirmed that this inhibitor possessed not only antiactivator activity but also antiplasmin activity, and hence they renamed this inhibitor α2-plasmin inhibitor. The antiactivator in the second antiactivator fraction in the present study is supposed to be the same inhibitor as α2-plasmin inhibitor and if this speculation is correct, it is also considered possible that the increase of α2-plasmin inhibitor in plasma may be related to Behçet's disease. In the present study, these theories are still open to speculation. On the other hand, there is the question of what the antiactivator in the first antiactivator fraction is. As shown in Fig. 2, while only a small amount of antiactivator which can inhibit both SK and UK in the same degree was eluted in the first antiactivator fraction in normal plasma, a great deal of anti-SK was eluted in the same first antiactivator fraction in patients' plasma. How should these results be interpreted? There is a possibility that the antiactivator whose affinity is markedly lower for UK than for SK may be increased in plasma of patients with Behçet's disease. There is another possibility, that an entirely new antiactivator which possesses the same molecular weight as the first antiactivator in normal plasma and can inhibit only SK may be increased in plasma of patients with Behçet's disease. The nature of this antiactivator, however, is still unclear. Further biochemical and biological investigations are underway in our laboratories.

As shown in Table 1, the two cases of allergic vasculitis showed a significant shortening of ELT. Although the fibrinogen content in the euglobulin fraction remained within the physiological range, a decreased plasminogen content was recognized in the euglobulin fraction in the second case and a decrease in the immediate type of antiplasmin content was recognized in the plasma of both cases. These results suggest that gradual activation of plasminogen may occur in plasma of patients with allergic vasculitis. Although the clinical definition of allergic vasculitis has yet to be established in the present study, the authors isolated two cases, which were diagnosed as allergic vasculitis according to Ruiter & Brandsma (1948), from other cutaneous vasculitis groups. The mechanisms producing allergic vasculitis as well as other cutaneous vasculitis are in most patients not adequately understood. Cochrane (1968) and Katz, Unanue & Dixon (1968) reported that antigen-antibody complexes deposited in small blood vessels induced excessive deposition of fibrin which might initiate vasculitis. Cunliffe (1968) and Menon, Cunliffe & Dewar (1969) reported that a decreased blood fibrinolytic activity might therefore favour the development of the lesions. On the other hand, many cases in which disseminated intravascular coagulation was induced in the course of allergic vasculitis have been reported (Robby *et al.*, 1973; Gola, 1976; Ito *et al.*, 1976). As the two cases in the present study failed to demonstrate any activation of coagulation factors, it was assumed that the significant shortening of ELT was not related to disseminated intravascular coagulation. However, it is not clear why the significant shortening of ELT was noticed in the two cases with allergic vasculitis. To clarify this problem the blood fibrinolytic enzyme of more cases should be investigated.

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Neutrophil chemotaxis in the presence of antibiotics: a re-evaluation using an agarose technique

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SUMMARY

Neutrophil chemotaxis in the presence of tetracycline and clindamycin was evaluated using an agarose technique. No significant inhibition of chemotaxis by these antibiotics could be demonstrated, in contrast to previously reported findings using the Boyden chamber technique for measurement of chemotaxis. Both agents caused a minor increase in movement towards a *P. acnes* derived chemotactic factor. Some reduction of chemokinesis by high concentrations of tetracycline was noted. No effect of the drugs upon generation of chemotactic activity from whole serum could be demonstrated. Normal cell movement occurred in the absence of extracellular calcium and magnesium, in contrast to reported findings using the Boyden chamber.

The effectiveness of certain antimicrobial agents in the treatment of acne vulgaris is well established, but their mode of action remains obscure. The clinical response of acne does not necessarily reflect the *in vitro* sensitivity of *P. acnes* to the antimicrobial agent used; penicillin, for example, is highly effective *in vitro* against most strains of this organism, but the drug is of little value in the treatment of acne. On the other hand, tetracycline, and to a lesser extent erythromycin, are clinically effective drugs, but they exert little influence upon the growth of *P. acnes* in the contents of comedones (Stoughton & Resh, 1976).

There is some evidence to suggest that in addition to their antibacterial effects, some antibiotics may have an anti-inflammatory action, particularly with regard to neutrophil accumulation. Potassium iodide-induced pustulation is inhibited by locally or systemically administered tetracycline and erythromycin (Plewig & Schopf, 1975). *In vitro* studies of the effect of antimicrobial agents upon neutrophil movement have also been performed (Majeski & Alexander, 1977; Esterly, Furey & Flanagan, 1978; Martin *et al.*, 1974), using the Boyden chamber technique for measuring cell movement through filters. These studies have provided interesting, but to some extent conflicting results.

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We have therefore evaluated the effect of tetracycline and clindamycin upon leukocyte chemotaxis using a different technique, the agarose system. We have also explored the possibility that chelation of calcium and magnesium by these antimicrobial agents might be of importance in altering chemotactic responses.

MATERIAL AND METHODS

Neutrophil chemotactic factors

Zymosan activated serum. Fresh human serum was incubated for 60 min at 37°C in the presence of zymosan (15 mg/ml). The reaction was terminated by heating at 56°C for 10 min; the serum was then centrifuged and frozen in aliquots. Heat inactivated serum was used as a control. N-formyl-L-methionyl-L-phenylalanine (N-f-Met-Phe) which is chemotactically active for human neutrophils was dissolved at 10 mM concentration in dimethyl sulphoxide, and then diluted in Hepes buffer containing 0·25% human serum albumin (HSA) to the required concentration (10^{-4} M or 2×10^{-5} M). Buffer containing HSA alone was used as a control solution. Dimethyl sulphoxide at this concentration was shown not to alter cell movement.

P. acnes derived chemotactic factor. P. acnes was grown in thioglycollate broth for 24 h; the culture was centrifuged and the supernatant divided into aliquots and frozen. Broth alone was used as the control solution.

Leukocytes

Six healthy adult donors were used. Thirty millilitres of heparinized blood (10 iu/ml) was drawn and allowed to sediment for 30 min in the presence of 1·5% dextran T-500. The leukocyte-rich supernatant was centrifuged at 150 g for 10 min, and the erythrocytes removed by hypotonic lysis. The leukocytes were harvested by further centrifugation at 150 g, followed by resuspension in Hepes buffer containing 0·25% HSA.

Assay

The method of Chenoweth, Rowe & Hugli (1979) was used, with minor modifications. Microscope slides (Van-Lab 48312-02, Van Waters & Rogers) were acid-alcohol washed, dipped in 0·5% gelatin solution, briefly rinsed in deionized water and air dried in a vertical position. Agarose was dissolved in deionized water at a concentration of 2 g/100 ml by heating, and cooled to 48°C in a waterbath. HSA (0·5 g/100 ml) was dissolved in double strength Hepes buffer (pH 7·45 at 37°C) at 48°C, and mixed with an equal volume of agarose solution. Different concentrations of antibiotics were incorporated into the gel at this stage. Five millilitres of this mixture was poured onto each slide; after setting, six groups of three wells, 3 mm diameter and 2·5 mm apart, were cut on each slide, using a template and punch. The slides were transferred to Petri dishes containing wet filter paper squares to provide humidification. Chemotaxis was measured by first placing chemotactic solution (10λ) in one outer well of each triplet, and the control solution in the other; neutrophil suspension (10λ) was then introduced into the centre well. The slide was then incubated for 2 h at 37°C in a humidified atmosphere. The slides were removed from the incubator and exposed to the atmosphere until the wells appeared dry. Fixation with methanol overnight was followed by careful removal of the gel, gentle washing with methanol and staining in Giemsa solution. Migration distances towards chemotactic and control wells were assessed using a microscope with an ocular graticule. The distance between the edge of the well and the furthest point at which three cells were aligned in the same plane, at right angles to the direction of migration, was measured. Figures are the number of graticule squares (10 squares = 0·75 mm). The difference between the active and control migration was taken as the chemotaxis distance. Migration distance refers to the total distance moved towards a well, in the studies of the effect of antibiotics upon zymosan activation of serum. Each test was performed in at least sextuplicate, on two or more slides. There were considerable differences between donors in the extent of migration which occurred.

The ability of tetracycline hydrochloride and clindamycin hydrochloride to alter the generation of chemotactic activity from fresh serum was examined as follows. Serum was divided into 1 ml aliquots, to which zymosan (15 mg/ml) was added. Antibiotics (clindamycin, 5 and 50 μg/ml, tetracycline hydrochloride 5 and 50 μg/ml) were added to half of the aliquots, prior to incubation of all the aliquots at 37°C for 60 min with agitation. At the end of the incubation period the reaction was terminated by heating at 56°C for 10 min. Antibiotics were added to the remaining aliquots at the end of the 1 h incubation at 37°C, followed immediately by heating to 56°C; these served as controls. Treated and control sera were compared in the chemotaxis assay by placing them in opposing wells across the same cell containing well: migration distances toward test and control wells from the same cell well were compared.

Chemokinesis

The effect of tetracycline in the gel upon serum-induced chemokinesis was examined as follows: single wells were cut in gels containing antibiotic at different concentrations (0, 2, 20 and 200 μg/ml). Cells were suspended in buffer containing 0, 2·5, 5 and 10% human serum. Cell migration after 2 h was measured in four different directions from each well and the mean results for six wells obtained for each serum and antibiotic concentration.

Thermal stability of antibiotics

In brief, the stability of clindamycin and tetracycline under the temperature conditions of the assay was confirmed as follows. Serial dilutions of the antibiotics, starting at 250 μg/ml, were made in duplicate tubes of thioglycollate broth. One tube of each pair was heated in a waterbath to 48°C and then allowed to cool to 37°C. *P. acnes* suspension was added to each tube, followed by incubation for 48 h at 37°C. For both antibiotics inhibition of bacterial growth occurred to the same antibiotic dilution in heated and unheated tubes, confirming stability of antibacterial activity at 48°C for a similar period to that to which the antibiotics were exposed in the preparation of the gels for the chemotaxis assay.

RESULTS

Addition of antibiotics to aggarose gel

Incorporation of tetracycline hydrochloride (2, 20 and 200 μg/ml) or clindamycin phosphate (1, 10 and 100 μg/ml) caused no significant reduction in neutrophil chemotaxis towards zymosan activated serum, N-f-Met-Phe or *P. acnes* derived chemotactic factors (Fig. 1a and b). There was a slight increase in movement towards the latter factor at 10 and 100 μg/ml clindamycin phosphate ($P<0·01$), and at 20 μg/ml tetracycline HCl ($P<0·05$). Tetracycline base and clindamycin hydrochloride were also tested and likewise caused no depression of chemotaxis. Random movement, which is small in this system, appeared unaltered by the presence of drugs.

Addition of antibiotics to cells

Suspension of cells in buffer containing HSA 0·25% with clindamycin hydrochloride (25 μg/ml) or

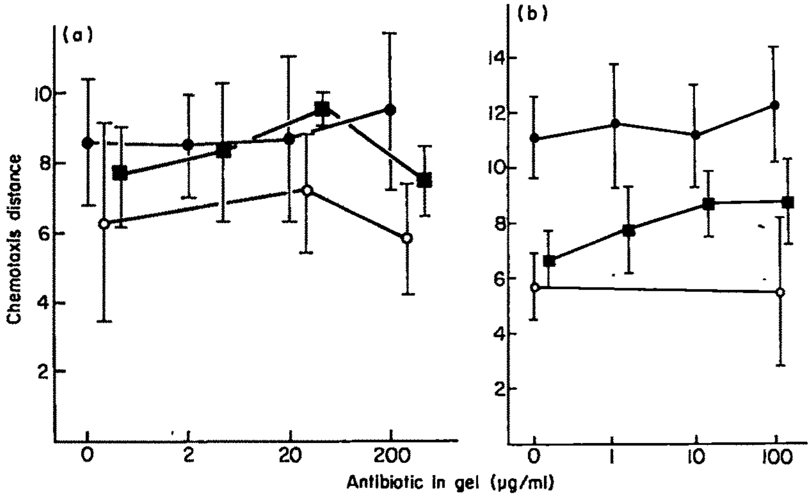

FIGURE 1. Chemotaxis with antibiotic incorporated in gel. (a) Tetracycline hydrochloride; (b) clindamycin phosphate. (●——●) ZAS; (■——■) *P. acnes* factor; (○——○) N-f-Met-Phe ((a) 2×10^{-5} M; (b) 10^{-4} M). Bars represent mean ± s.d.

tetracycline hydrochloride (25 µg/ml) did not depress chemotaxis toward ZAS or N-f-Met-Phe (Fig. 2).

Addition of antibiotics to chemoattractants

Clindamycin hydrochloride and tetracycline hydrochloride were added to ZAS and N-f-Met-Phe prior to introduction into the wells, giving a final concentration of 200 µg/ml. In the control wells, ZAS and N-f-Met-Phe to which an equivalent volume of buffer alone was used: migration of cells towards the two wells was compared. There was a slight but not significant increase in mobility towards the attractants containing antibiotics (Fig. 3).

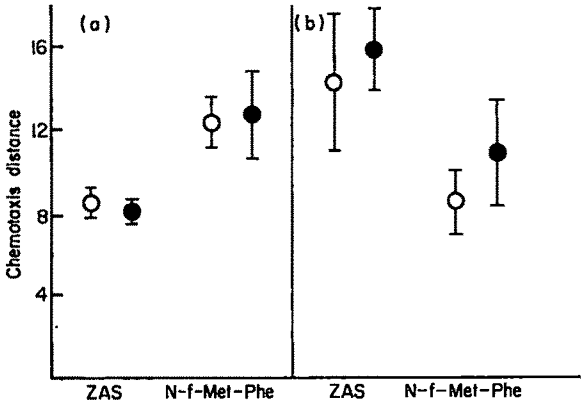

FIGURE 2. Chemotaxis with antibiotic incorporated in cell suspending medium. (○) Buffer; (●) clindamycin hydrochloride 25 µg/ml (a); tetracycline hydrochloride 25 µg/ml (b). Bars represent mean ± s.d.

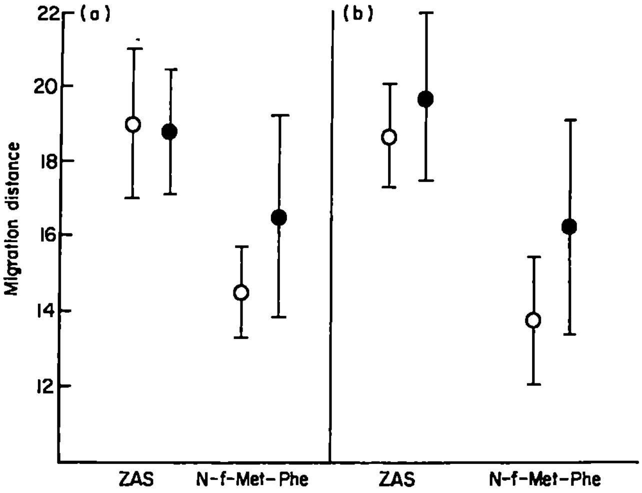

FIGURE 3. Chemotaxis with antibiotic added to attractant. (○) Attractant alone; (●) attractant + clindamycin hydrochloride 200 μg/ml (a); attractant + tetracycline hydrochloride 200 μg/ml (b). Bars represent mean ± s.d.

Addition of antibiotics to serum prior to zymosan activation

Movement of cells towards serum activated with zymosan in the presence of antibiotics (tetracycline hydrochloride and clindamycin hydrochloride 5 and 50 μg/ml) was compared with movement towards zymosan activated serum (ZAS) to which the antibiotics were added at the end of the activation period, as a control. No difference was seen between migration towards the two types of ZAS preparations (Fig. 4).

Effect of tetracycline upon chemokinesis.

Addition of 10% human serum to the buffer in which the cell suspension was made caused increased random migration of cells (Fig. 5). The presence of tetracycline hydrochloride in the gel at concentra-

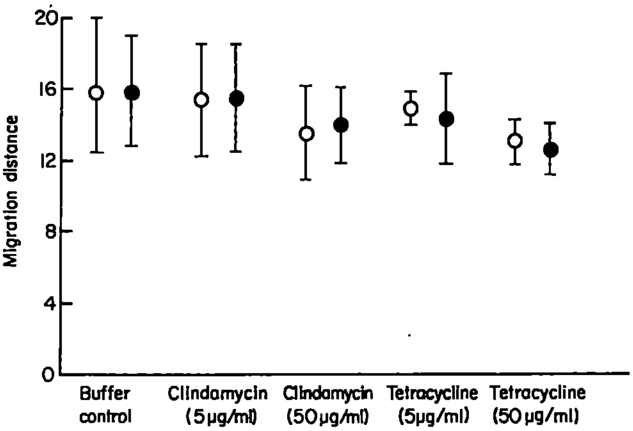

FIGURE 4. Effect of antibiotics upon activation of serum by zymosan. (○) Antibiotic added after zymosan activation (control); (●) antibiotic added before zymosan activation (test). Bars represent mean ± s.d.

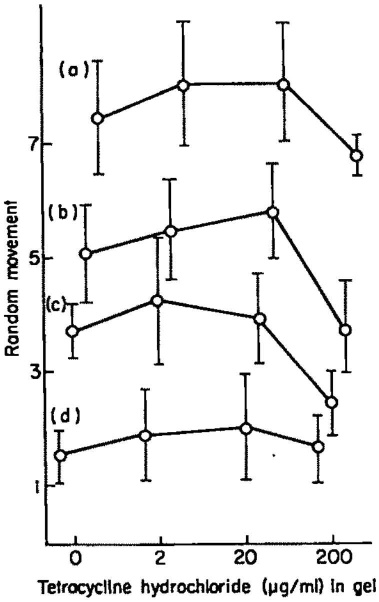

FIGURE 5. Random migration of cells. Effect of serum in cell suspending medium and tetracycline in the gel. (a) 10% serum; (b) 5% serum, $P<0{\cdot}01$; (c) 2·5% serum, $P<0{\cdot}0025$; (d) 0% serum. Bars represent mean ± s.d.

tions of 2 and 20 μg/ml did not alter the degree of random movement occurring in the presence of 10% human serum. At 100 μg/ml random movement induced by the addition of lower concentrations of serum was significantly decreased (2·5% serum, $P<0{\cdot}0025$; 5% serum, $P<0{\cdot}01$). The greater increase in random movement caused by 10% serum was not significantly depressed. Random movement occurring in the absence of serum remained unaltered by tetracycline.

Effect of reduction of divalent cation concentration in the medium

Gels were prepared using buffer without calcium, without magnesium and without either cation present. Chemotaxis toward ZAS and toward N-f-Met-Phe was not altered by the absence of either

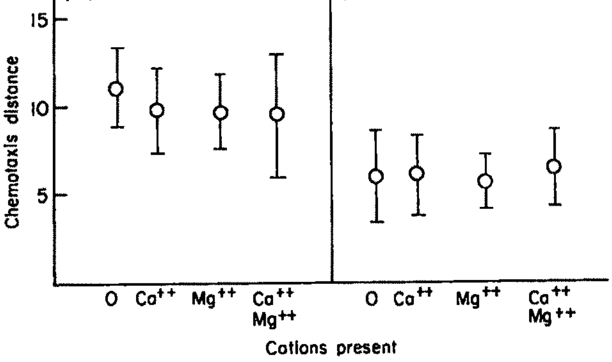

FIGURE 6. Effect of altering levels of divalent cations in the chemotaxis system. Movement towards ZAS and N-f-Met-Phe. (a) ZAS; (b) N-f-Met-Phe. Bars represent mean ± s.d.

or both cations (Fig. 6). Addition of disodium ethylenediaminetetracetate (diosodium EDTA) to the gel in concentrations of up to 200 μg/ml was also without effect upon chemotaxis towards ZAS and N-f-Met-Phe. However, when disodium EDTA (30 μg/ml) was added to the Ca^{++} and Mg^{++} free gel, cell suspension and ZAS, chemotaxis was reduced (4·9±2·4; 8·7±1·5 in the absence of EDTA). The density of the fan of migrating cells was also noted to be less.

DISCUSSION

Previous studies demonstrating inhibition of neutrophil chemotaxis by antibiotics (Majeski & Alexander, 1977; Esterly *et al.*, 1978; Martin *et al.*, 1974) were performed using the Boyden chamber, which measures neutrophil movement through filters. This is a well established technique which has provided valuable information regarding neutrophil movement. More recently (Nelson, Quie & Simmons, 1975), a method for examining chemotaxis under agarose has become available, the results of which largely correlate well with other methods. This method was employed in this present study in an attempt to evaluate the differing results obtained in previous studies, with regard to dose-response relationships and alteration in random movement. We also wished to evaluate the effect of alteration in concentrations of divalent cations; both tetracycline and clindamycin are chelating agents, and alteration in the availability of calcium and magnesium has not been previously explored as a mode of action of these drugs in their role as possible anti-inflammatory agents.

Using this method, we were unable to confirm an inhibiting effect of tetracycline and clindamycin upon neutrophil chemotaxis, when the agents were incorporated in the gel, in the cell suspension model or mixed with the chemoattractants. Furthermore, we were unable to confirm the conclusions of Majeski & Alexander (1977) that tetracycline altered the generation of chemoattractant activity in serum by complement activating agents, either by inhibiting generation or by inactivation of the reaction product. The agarose system particularly lends itself to such an application, as cell migration from a single cell well towards wells containing treated and control attractants can be compared directly in the same plate.

Majeski & Alexander (1977), using eight different tetracycline preparations, found maximal inhibition of chemotaxis at antibiotic concentrations of 100 and 1000 μg/ml, in contrast to the findings of Esterly *et al.* (1978), who found maximum inhibition occurred at the range of 0·01–10 μg/ml, close to the therapeutic range, with normal chemotaxis at 300 μg/ml (almost identical findings occurred with erythromycin base and clindamycin). These findings are similar to those observed by Martin *et al.* (1974); however, the latter study may not be comparable, as mycoplasma antigen was used as the chemoattractant. Random movement was found to be altered in parallel with the chemotactic response, which contrasts with the more recent findings of Esterly *et al.* (1978), who found normal random movement at all antibiotic concentrations tested.

Cell movement through the pores of a filter and along an interface between glass and gel may be very different; this is suggested by the lack of dependence upon the addition of Ca^{++} and Mg^{++} to the suspending medium which we have demonstrated in the agarose system, for chemotaxis towards both ZAS and N-f-Met-Phe, and which contrasts with reported findings using the Boyden chamber technique (Wilkinson, 1974; Gallin & Rosenthal, 1974). Alterations in membrane permeability to Ca^{++} and in membrane bound Ca^{++} levels do occur as a result of neutrophil chemoattractant interaction (Naccache *et al.*, 1977, 1979) but it was not clear from the latter studies whether a net influx of Ca^{++} was necessary for the chemotactic activity of neutrophils. The adherence of neutrophils to untreated glass slides has been shown to be unaltered by omission of calcium and magnesium from the suspending medium (Wayne-Smith *et al.*, 1979); however, adherence to serum treated slides was diminished in the absence of magnesium. The divalent cation requirement of a chemotaxis system may therefore to some extent be determined by the character of the interface along which cell migration occurs. Since the addition of disodium EDTA to the cell suspension, the gel and ZAS simultaneously does reduce chemotaxis in the absence of added Ca^{++} and Mg^{++}, it is possible trace amounts of these cations may be a requirement of the system; further studies would be necessary to verify this.

The Boyden chamber and agarose methods have also provided contrasting results in patients suffering from infections (Frei, Hermanovicz & Pecoud, 1978). Using small pored filters, defective chemotaxis of neutrophils from infected patients was observed; when larger pored filters or the agarose method was employed, normal chemotaxis occurred. Deformability of neutrophils is a prerequisite for movement through filters and may be reduced when a substantial proportion of cells are immature, for example in infected patients. The deformability requirement for movement along a glass–agarose interface is likely to be less.

Which *in vitro* system approximates more closely to *in vivo* situations is debatable. In the present study, we have attempted to minimize the possible artefacts to which any *in vitro* method is heir, by employing three different types of chemoattractants, and by introducing the antimicrobial agents under investigation into the system in different ways.

While an anti-inflammatory effect of antimicrobial agents modified through modification of chemotaxis offers an attractive hypothesis for certain of their clinical effects, use of an agarose method to evaluate this has not provided *in vitro* support for such an action. Cell movement through filters does appear to be altered by certain antibiotics, but not necessarily at drug levels which would be obtained *in vivo* (Majeski & Alexander, 1977). A reliable method for evaluation of neutrophil chemotaxis *in vivo* would be a valuable contribution in this area.

ACKNOWLEDGMENT

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Localization of non-specific esterase and endogenous peroxidase in the murine epidermal Langerhans' cells

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SUMMARY

The localization of the enzymatic activity of non-specific esterase and endogenous peroxidase of the Langerhans' cells in murine epidermal sheets separated with EDTA was observed by means of light and electron microscopy. The Langerhans' cells exhibited non-specific esterase activity in the outer portion of the plasma membrane and endogenous peroxidase activity in the nuclear envelope and the endoplasmic reticulum.

Non-specific esterase activity of murine epidermal dendritic cells has already been described (Jarrett & Riley, 1963). However, so far as we know, no studies have been made to ascertain the ultrastructural identification of the non-specific esterase positive dendritic cells as Langerhans' cells, and to reveal the localization of endogenous peroxidase in the Langerhans' cells. The present work was undertaken to examine the localization of non-specific esterase and endogenous peroxidase activity of Langerhans' cells using EDTA-separated epidermal sheets of mouse ears.

MATERIALS AND METHODS

The auricles of 5-week-old C57BL mice were used.

Separation of epidermis

The excised auricles were immersed for 1 h in EDTA (Juhlin & Shelley, 1977). After this treatment the epidermis of the inner side of the auricles was separated from the dermis with fine forceps.

Fixation

The separated epidermal sheets were fixed in 1·5% aqueous glutaraldehyde with 0·05 M cacodylate buffer at pH 7·4 and 1% sucrose for 5 min at 4°C.

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Incubation

Non-specific esterase. The medium was 10 mg α-naphthyl acetate in 0·4 ml acetone, 2·4 ml hexazotized pararosanilin in 40 ml of 0·1 M phosphate buffer at pH 7·4 and adjusted to pH 7·0 by 2 N NaOH solution. Incubation time was 3 min at room temperature.

Endogenous peroxidase. The medium was 25 mg of 3,3-diaminobenzidine- 4 HCl in 50 ml of 0·1 M tris-HCl buffer at pH 7·4 containing 3·5 g sucrose. Incubation time was 1 h at 25°C followed by 1 h post-incubation in the same volume of the medium containing 0·03 ml of 35% H_2O_2 (Daems, Koerten & Soranzo, 1976).

Microscope studies

For the light microscope study incubated epidermal sheets were mounted with glycerine jelly. For electron microscopic examination, incubated materials were post-fixed with 2% OsO_4 in 0·2 M phosphate buffer at pH 7·4, dehydrated in a graduated series of ethanols, immersed in propylene oxide, and embedded in epon 812. Ultra-thin sections were cut on a Porter Blum ultramicrotome. The obtained sections, both stained and unstained with uranyl acetate and lead citrate, were observed in a Hitachi HS-7S electron microscope.

RESULTS

Light microscopy demonstrated regularly distributed non-specific esterase positive dendritic cells all over the EDTA-separated murine epidermal sheets which had been incubated for non-specific esterase activity (Fig. 1). The epidermal sheets incubated for endogenous peroxidase activity gave very faint reactions. However, unstained 1 μm thick sections of epon embedded materials revealed positive reactions on the nuclear membrane of some cells. By electron microscopy of the unstained sections, the reaction products for non-specific esterase activity were localized uniformly on the plasma membrane, but there was little in the cytoplasm (Fig. 2a). The reaction products of endogenous peroxidase activity were on the nuclear envelope and the endoplasmic reticulum (Fig. 2b). It was confirmed,

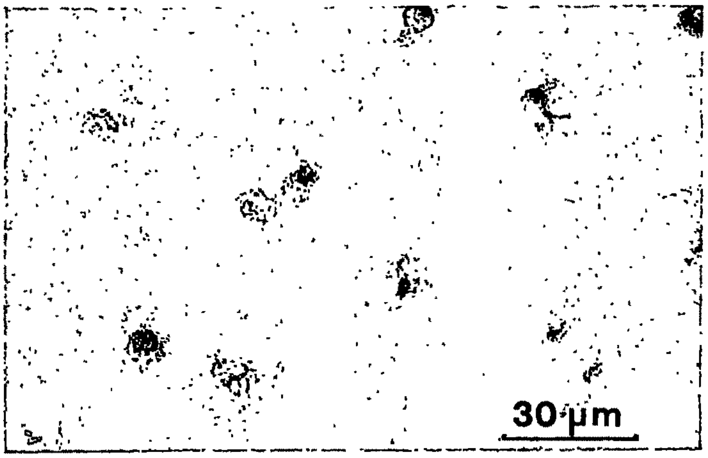

FIGURE 1. Photomicrograph of murine epidermal sheet separated with EDTA and incubated for non-specific esterase activity, showing the distribution of the positive dendritic cells.

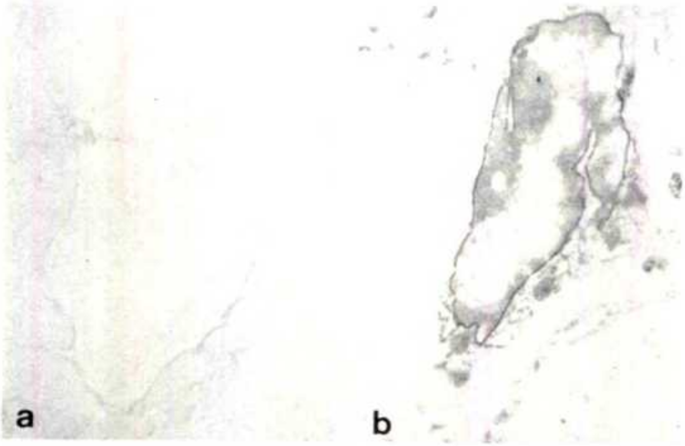

FIGURE 2. (a) Electron micrograph of a murine epidermal non-specific esterase positive cell (unstained). Reaction products are confined to plasma membrane (× 2400). (b) Electron micrograph of a murine epidermal endogenous peroxidase positive cell (unstained). Reaction products are confined to the nuclear envelope and the endoplasmic reticulum (× 4800).

in uranyl acetate and lead citrate stained sections, that the non-specific esterase reaction products were on the outer aspect of the plasma membrane. The non-specific esterase positive cells possessed Langerhans' cell granules (Fig. 3), as did the endogenous peroxidase positive cells (Fig. 4).

DISCUSSION

Mackenzie & Squier (1975) pointed out that the EDTA-separated epidermal sheets had advantages over sodium bromide separated sheets, for histochemical (ATPase) and ultrastructural investigation of epidermal Langerhans' cells. Therefore, we employed the former method to examine the localization of non-specific esterase and endogenous peroxidase, and had good results. Evidence for analogous functions of Langerhans' cells and macrophages have been reported recently (Stingl *et al.*, 1977; Rowden, 1977). Jaubert *et al.* (1978) published an ultrastructural demonstration of the localization of non-specific esterase on human lung macrophages. According to their results, the reaction products were confined to the outer aspect of the plasma membrane. The results of our investigation of Langerhans' cells are in agreement with these workers. On the other hand, Daems *et al.* (1976) described murine peritoneal tissue macrophages as having endogenous peroxidase activity in the nuclear envelope and the endoplasmic reticulum in contrast to monocyte derived macrophages which were positive in the granules. Wisse (1974) demonstrated the endogenous peroxidase activity of the Kupffer cells in murine liver localized in the nuclear envelope and the endoplasmic reticulum.

In the present examination, the enzymic localization in murine epidermal Langerhans' cells was equivalent to that of, as Daems *et al.* (1976) called them, 'tissue macrophages'. However, a functional similarity between macrophages and Langerhans' cells remains to be confirmed, as the phagocytic activity of Langerhans' cells is not as great as that of macrophages (Wolff & Schreiner, 1970).

Furthermore, the antigen trapping capacity of Langerhans' cells is different from that of macrophages but parallel to non-phagocytic reticulo-endothelial cells in lymph nodes (Shelley & Juhlin, 1976).

Wolff & Schreiner (1970) and Hashimoto (1971) did not find endogenous peroxidase activity in human and guinea-pig Langerhans' cells. However, we conclude that perinuclear peroxidase activity

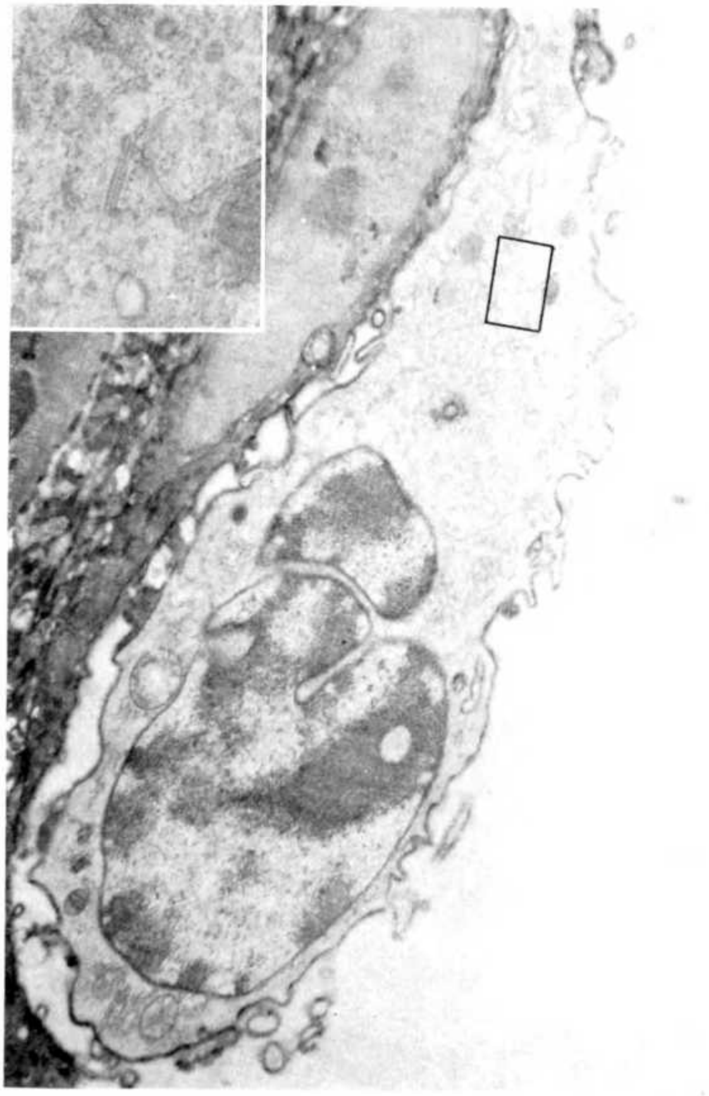

FIGURE 3. Electron micrograph of EDTA-separated murine epidermis showing positive non-specific esterase activity of the Langerhans' cell (stained with uranyl acetate and lead citrate). Reaction products are confined to the outer aspect of the plasma membrane of the Langerhans' cell (× 12,000). Inset shows Langerhans' cell granule (× 40,000).

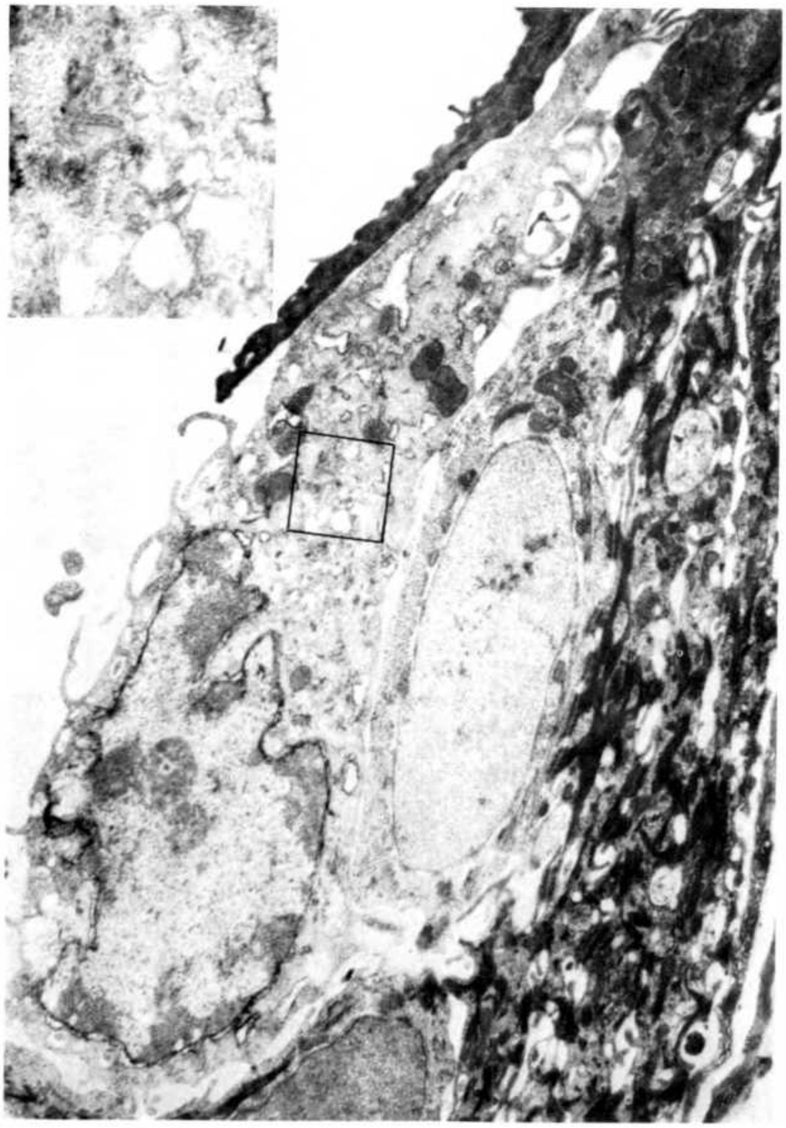

FIGURE 4. Electron micrograph of EDTA-separated murine epidermis showing positive endogenous peroxidase activity of the Langerhans' cell (stained with uranyl acetate and lead citrate). Reaction products are confined to the nuclear envelope and the endoplasmic reticulum (× 9600). Inset shows Langerhans' cell granules (× 48,000).

of Langerhans' cells is very weak and will be inactivated by fixation for several hours, as our positive results were obtained with fixation of 5 min in contrast to the 2 or 3 h of other authors.

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In situ identification and enumeration of T lymphocytes in cutaneous T-cell lymphomas by demonstration of granular activity of acid non-specific esterase

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SUMMARY

Activity of acid non-specific esterase (ANAE) in infiltrating lymphocytes in nine patients with mycosis fungoides and four patients with parapsoriasis en plaques was examined in frozen tissue sections. Within the dermal infiltrate 81·7±6·1% of all lymphocytes contain this enzyme in mycosis fungoides, 74·7±3·3% in parapsoriasis en plaques. These results correlate well with the percentage of T lymphocytes determined by immunological techniques. Mycosis fungoides may be regarded as an ANAE-positive cutaneous T-cell lymphoma. Because of its simplicity this technique may be of value in further investigations of mycosis fungoides.

In this study we investigated whether neoplastic T lymphocytes can be identified and enumerated reproducibly in tissue sections of patients suffering from parapsoriasis en plaques and mycosis fungoides, using acid non-specific alpha-naphthyl acetate esterase (ANAE) as a T-cell marker.

The *in situ* differentiation of T lymphocytes in tissue sections is difficult. Brubaker & Whiteside (1977) reported a modified technique of spontaneous rosette formation with sheep erythrocytes (E rosettes) for this purpose. Another possibility for the *in situ* identification of T lymphocytes is the demonstration of human T-lymphocyte specific antigen (HTLA) (Smith *et al.*, 1973) by peroxidase-labelled antisera. This method has been applied to the *in situ* identification of T lymphocytes in mycosis fungoides using indirect immunofluorescence (Braathen, Førre & Natvig, 1979) or indirect immunoperoxidase staining techniques (Chu & MacDonald, 1979). These methods, however, cannot be performed generally, because HTLA antisera can be prepared only in highly specialized laboratories.

While the classic methods for the differentiation of T and B lymphocytes depend on membrane

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properties, Mueller *et al.* (1975) found that the activity of lysosomal acid alpha-naphthyl acetate esterase (ANAE) represented a good marker for T lymphocytes in mice. Ranki, Tötterman & Häyry (1976a) confirmed these findings, but demonstrated that stimulated T lymphocytes do not exhibit ANAE-activity in all instances.

In the last 3 years the presence of ANAE-activity in resting human T lymphocytes has been established in various studies (Ranki, Tötterman & Häyry, 1976b; Kulenkampff, Janossy & Greaves, 1977; Sher, Fripp & Wadee, 1977; Knowles *et al.*, 1978; Obrist, Albrecht & Nagel, 1978). However, the analysis of stimulated T lymphocytes revealed that only Con A-stimulated lymphocytes keep their ANAE activity, while after PWM and PHA stimulation not all T lymphoblasts exhibited ANAE-activity (Ranki *et al.*, 1976b; Kulenkampff *et al.*, 1977; Tötterman, Ranki & Häyry, 1977; Knowles *et al.*, 1978).

Investigating various malignant cell lines, Sundström *et al.* (1978) demonstrated the presence of ANAE activity in T-lymphoma cell lines, but also in some B-lymphoma cell lines. The authors concluded that the presence of ANAE activity cannot be regarded as T specific when malignant lymphoid cells are considered.

Histochemical studies of malignant cutaneous lymphomas have been few. Hovmark (1977) found in one patient with parapsoriasis en plaques more than 85% of the infiltrating lymphocytes carrying the ANAE marker. Two cases of lymphoblastic cutaneous lymphomas showed 45% ANAE positive lymphocytes in one case, 100% in the other (Knowles & Holck, 1978).

MATERIALS AND METHODS

Patients

Skin biopsies of four patients with parapsoriasis en plaques and of nine patients with mycosis fungoides were taken under local anaesthesia (Scandicain 1%) prior to any therapy. Of the patients with mycosis fungoides two were in the erythematous stage, five in the infiltrative stage and two in the tumorous stage of the disease.

Methods

The demonstration of ANAE was performed on formol sucrose prefixed 8 μm cryostat sections exactly as described by Mueller *et al.* (1975).

Lymphocytes within tissue sections were regarded as positive for ANAE when they exhibited one or more brown granular deposits of the reaction product within their cytoplasm. Enumeration of ANAE-positive lymphocytes was carried out at high magnification with light microscopy. ANAE-negative lymphocytes were differentiated morphologically from other enzyme-negative cells within the infiltrate. A round to oval nucleus and only scanty cytoplasm were considered to be lymphocyte characteristics. The differentiation from monocytes and histiocytes, which may be impossible in routine stains, is simple as these cells show strong diffuse enzyme activity. All lymphocytes within one section were counted.

RESULTS

The cellular infiltrate in parapsoriasis en plaques and mycosis fungoides is situated in the upper and middle corium (Fig. 1), showing distinct lymphocytic epidermotropism (Fig. 2). Within most lymphocytes a strong dot-like reaction of ANAE can be observed. The reaction product is localized in granules measuring from 0·6 to 2·0 μm. These granules are found in the periphery of the cytoplasm, usually one to three granules per lymphocyte: T pattern of ANAE-activity (Kulenkampff *et al.*, 1977).

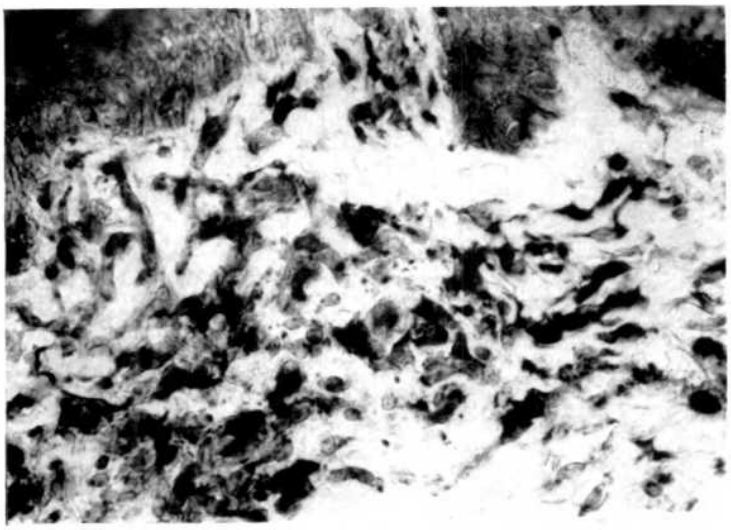

FIGURE 1. Subepidermal infiltrate in mycosis fungoides. Monocytes and macrophages show diffuse reaction of ANAE (M pattern), while most lymphocytes exhibit a granular localization of the reaction product (T pattern) (× 300).

Macrophages exhibited a strong diffuse activity of ANAE in the whole cytoplasm (Fig. 1): M pattern of ANAE activity.

The *in situ* enumeration of ANAE-positive lymphocytes within the infiltrate gave a percentage of $74{\cdot}7 \pm 3{\cdot}3\%$ in parapsoriasis en plaques and of $81{\cdot}7 \pm 6{\cdot}1\%$ in mycosis fungoides (Fig. 3). The difference between the percentage in parapsoriasis en plaques and mycosis fungoides is, however, not statistically significant (Wilcoxon test). The clinical stage of mycosis fungoides did not correlate with the percentage of ANAE-positive lymphocytes.

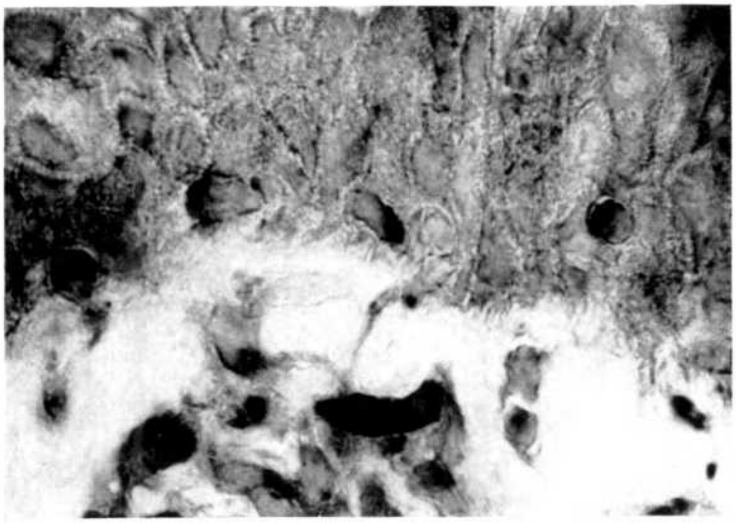

FIGURE 2. Lymphocytes with strong dot-like ANAE activity infiltrating into the epidermis (× 500).

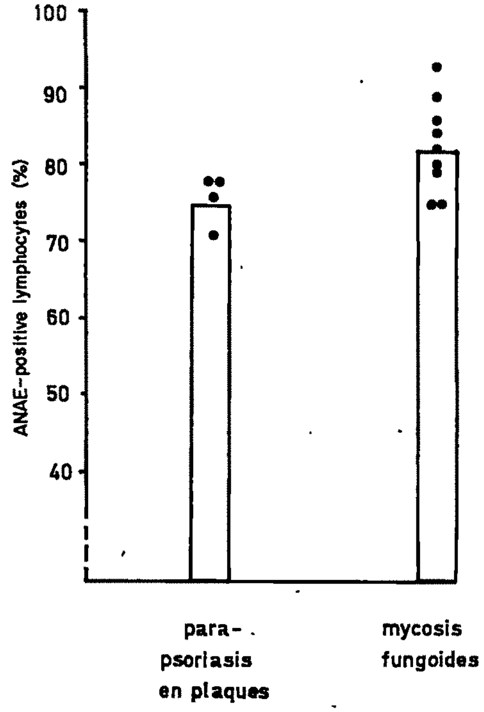

FIGURE 3. Percentage of ANAE-positive lymphocytes in tissue sections in parapsoriasis en plaques and mycosis fungoides.

DISCUSSION

The low pH optimum and the granular cytoplasmic localization suggest a lysosomal origin of acid alpha-naphthyl acetate esterase, although the granules appear larger than a single lysosome. A well known characteristic of T lymphocytes is the presence of aggregated lysosomes (Watanabe *et al.*, 1974). The size of these lysosomal aggregates correlates well with the size of the granular reaction product, so that one can assume that ANAE is localized in these structures.

Mycosis fungoides is a malignant T-cell lymphoma of probable monoclonal origin (Edelson *et al.*, 1979). Parapsoriasis en plaques is regarded as a possible precursor disease of mycosis fungoides. The enzyme-cytochemical properties of lymphocytes belonging to such an infiltrating malignant clone should be identical, either exhibiting activity of a certain enzyme or not. Such identical behaviour is confirmed by our results. In each of the nine patients with mycosis fungoides most of the dermal infiltrating lymphocytes (81·7±6·1%) show ANAE activity of the T pattern. No instances of ANAE-negative mycosis fungoides have been observed.

The enumeration of T lymphocytes by application of other methods to the infiltrate of mycosis fungoides has revealed different results. Spontaneous erythrocyte-rosetting was observed in 94% liberated viable lymphocytes by Edelson *et al.* (1974), but only in 44% by Burg *et al.* (1978). Using anti-T-cell globulin, however, Burg *et al.* (1978) found that 80% of the liberated lymphocytes carried this T-cell marker. Braathen *et al.* (1979) demonstrated by indirect anti-HTLA immunofluorescence studies 65–80% positive lymphocytes in the dermal infiltrate in mycosis fungoides. These results are in good agreement with 81·7% positive lymphocytes in our study. The similar percentage of T lymphocytes, using HTLA or ANAE activity as a T-cell marker, permits the conclusion that granular

ANAE activity is a sensitive and reliable marker for the identification of T-lymphocytes in parapsoriasis en plaques and mycosis fungoides. If a proportion of the involved T-lymphocytes in mycosis fungoides have undergone blast transformation, they nevertheless seem to have retained ANAE activity in the cases we have studied.

Because of the membrane localization of HTLA its demonstration by immunoperoxidase techniques is particularly valuable in ultrastructural studies. The simplicity of technique for the demonstration of ANAE activity suggests that enzyme cytochemistry may be the method of choice in light microscopic studies of cutaneous T-cell lymphomas.

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Sex-linked ichthyosis and placental sulphatase C deficiency

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SUMMARY

Ichthyosis was diagnosed in six boys born from pregnancies in which placental steroid sulphatase deficiency was present. In four cases the diagnosis of sex-linked ichthyosis was certain, in the other two the ichthyosis was probably of the sex-linked type.

No arylsulphatase C activity could be demonstrated histochemically either in the epidermis of these boys, or in the epidermis of adults with sex-linked ichthyosis, whilst it was present in normal controls. In cultured skin fibroblasts steroid-sulphatase activity was extremely low. We conclude that deficiency of steroid sulphatase C or arylsulphatase C or a third functionally linked enzyme is an important factor in the pathogenesis of sex-linked ichthyosis.

Three different types of arylsulphatases have been described: two lysosomal enzymes—arylsulphatase A and arylsulphatase B—and one microsomal—arylsulphatase C (Farooqui & Mandel, 1977). Furthermore three steroid sulphatases are known; these enzymes are very similar to the arylsulphatases, but probably not identical (Lakshmi & Balasubramanian, 1979; Hameister *et al.*, 1979).

Arylsulphatases A and B have been reported to be normally present in the skin (Sachs & Braun-Falco, 1960). Arylsulphatase C activity can be demonstrated histochemically in the granular layer of the normal epidermis and in the inner sheath of the hair follicles (Koudstaal, 1975). Its function has not been known so far (Roy, 1977). These three enzymes are not only present in the skin, but also in various other organs including the placenta.

Deficiency of arylsulphatase A causes a serious neurological syndrome (metachromatic leukodystrophy); deficiency of arylsulphatase B causes a mucopolysaccharidosis (Maroteaux-Lamy). No skin symptoms have been reported in these disorders. Deficiency of all three arylsulphatases (multiple

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sulphatase deficiency) is an extremely rare condition with severe neurological symptoms. The skin in this multiple deficiency has been reported to show ichthyosis vulgaris in three out of the four published cases of both sexes (Rampini *et al.*, 1970; Austin, 1973). In the normal placenta steroid sulphatase and arylsulphatase activity is present. Clinical significance of placental steroid sulphatase deficiency was first established in a sex-linked obstetric anomaly (France & Liggins, 1969). In this disorder steroid sulphatase C and arylsulphatase C activity are both virtually absent in the placenta (France, Seddon & Liggins, 1973). Until recently the only known symptom of placental sulphatase deficiency has been a failure of the oestrogen level in the maternal urine to rise during pregnancy. In the second half of normal pregnancies this level rises sharply; the oestrogens are derived from sulphated steroids produced by the fetal adrenal glands and the fetal liver. In the transformation of these precursors into oestrogens they are first desulphated in the placenta, for which process the presence of steroid sulphatase is necessary. Hereafter passage into the maternal blood stream is possible. In steroid sulphatase deficient placentas desulphation being impossible no oestrogens are formed and so the level of this hormone in the maternal urine fails to rise.

The placenta being a part of the child, placental sulphatase is a fetal enzyme. As in all known cases of placental sulphatase deficiency a boy has been born, sex-linkage is strongly suggested (France & Liggins, 1969; Cedard *et al.*, 1971; Fliegner, Schindler & Brown, 1972; France *et al.*, 1973; Oakey, Cawood & McDonald, 1974; Tabei & Heinrichs, 1976).

MATERIALS AND METHODS

In the histochemical reaction bromonaphthylsulphate was used as a substrate. In the presence of an azo-dye after desulphation of the substrate a red coloured naphthyl-azo-salt was precipitated. Details of this technique and of the biochemical background are given by Jöbsis *et al.* (1980) and by van der Loos *et al.* (1979).

Biochemical methods were used on placenta and on skin fibroblasts. Arylsulphatase C was estimated in placental microsomes obtained by differential ultracentrifugation (105,000 *g* fraction). The method of Murphy *et al.* (1971) was applied. Steroid sulphatase in placental microsomes was estimated according to Osathanondh *et al.* (1976).

Steroid sulphatase activity in cultured fibroblasts was measured in 10^6 cells, harvested after trypsin treatment and treated by freezing-thawing.

Incubation with 10 μM dehydroepiandrosterone sulphate ($10^5 \times$ dpm ^{3}H) in 100 mM Tris-HCl buffer, pH 7·8 was carried out for 4 h. Free dehydroepiandrosterone was estimated after toluene extraction by liquid scintillation counting.

CASE HISTORIES

In the Netherlands steroid sulphatase deficiency has been diagnosed in six pregnancies. All six boys born from these pregnancies have developed an ichthyotic condition. Cases 1, 2, 3 and 5 have been examined by one of us (dG), Cases 4 and 6 by dermatologists elsewhere.

Case 1

A boy born at term from a pregnancy in which the oestrogens in the urine of the mother had remained extremely low. At birth his general condition was excellent. The skin of his cheeks, nose, breast and hands showed a fine scaling. When 6 weeks old, large white scales adherent in the centre appeared on his scalp, front and breast. Smaller scales developed on the back especially in the median area. The arms and legs showed a very fine white scaling. The hands, feet and thighs were normal. At 4 months there was a fully developed ichthyosis on the scalp, trunk and extremities, sparing the axillae, antecubital and popliteal fossae and the genital area. At 2 years during a hot summer he showed only slight scaling. His mother had a normal skin, but her brother, who had died after an accident, was stated to have had an ichthyotic skin. In the family of the mother's mother more males were known with the same skin condition. The appearance of the skin, the early onset, and the family history were consistent with sex-linked ichthyosis.

Case 2

A boy delivered by caesarean section. The oestrogen level in the mother's urine had been extremely low. The general condition of the boy was bad but he recovered rapidly. At birth his skin was described as dry with fine scaling. At 2 weeks, his scalp, temporal areas and cheeks, breast, upper back and extensor surfaces of the legs showed white ichthyotic scales. The scales on the scalp were very large; his hair was normal. The skin of his mother had been normal, but two of her brothers were known to suffer from ichthyosis. The appearance of the skin, the early onset and the family history were in accordance with sex-linked ichthyosis.

Case 3

A boy, also born from a pregnancy with an extremely low oestrogen level, was normal at birth. When 6 months old his legs showed brown ichthyotic scaling, sparing the knee folds. His cheeks were dry, and his back showed fine white scaling. Other areas were free.

At 1 year the front of his trunk and to a still lesser degree his back showed slight scaling. His legs showed the same brown ichthyotic scaling as previously noticed (Fig. 1). At 2½ years the condition had not changed. There was no follicular keratosis, and the palms and soles were normal. The mother had a dry skin, the skin of the father was normal, and no other cases were known in the family.

The diagnosis of sex-linked ichthyosis has not been established with absolute certainty. Undoubtedly there was an ichthyotic condition on the legs as in sex-linked ichthyosis; however, the discrepancy between the heavy scaling on the legs and the slight symptoms on the other parts is unusual.

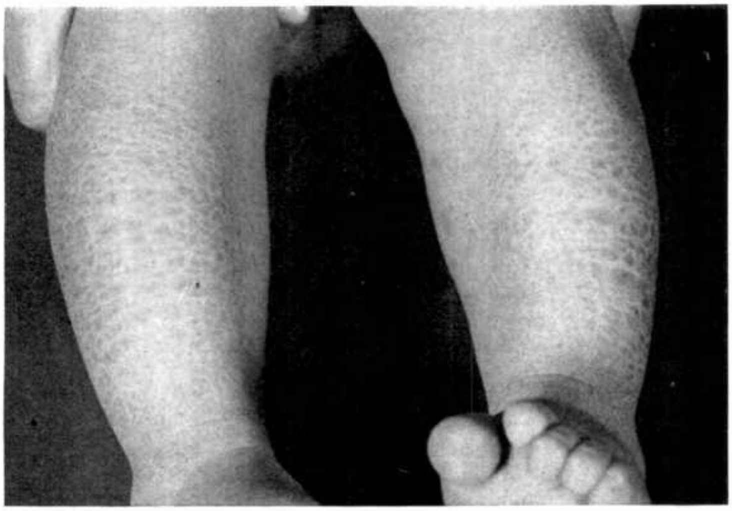

FIGURE 1. Case 3. Legs showing ichthyotic scaling.

The early onset and the size and colour of the scales on the legs are in accordance with sex-linked ichthyosis. Cases 1, 2 and 3 have been briefly recorded by Koppe *et al.* (1978).

Elsewhere in The Netherlands three other pregnancies with placental steroid sulphatase deficiency have been observed; of these we examined Case 5 at the age of 1 year.

Case 4

A boy. Ichthyosis was noticed shortly after birth. His only brother also had ichthyosis. Their mother had a dry skin, the skin of their father was normal. No other affected individuals were known in the family. In both brothers ichthyosis was certainly present, the diagnosis of sex-linked ichthyosis being more likely than dominant ichthyosis vulgaris.

Case 5

A boy. A few days after birth ichthyosis was noted. This condition subsided gradually but then remained stationary. At 1 year the lower part of the abdomen and the lateral aspect of his ankles and adjacent skin of the legs showed brown ichthyotic scales. Fine white scales were present on his scalp. Other parts of the skin were clear. His father had normal skin. The legs of his mother showed a mild ichthyotic condition, especially on the shins; she stated that her mother had a similar condition. No other cases were known in the family. Although the ichthyosis was restricted to a small part of the body, the early onset, the brown colour and the appearance of the scales and the condition in the mother were in accordance with sex-linked ichthyosis.

Case 6

A boy, born with a fine scaling over the whole body. On examination at the age of 16 months both legs showed large brown ichthyosiform scales sparing the popliteal fossae. On his body and arms fine whitish scales were present sparing the folds. In the family no one was known with a dry skin. Sex-linked ichthyosis was more likely than dominant ichthyosis for the same reasons as in Case 3.

Until now we have not come upon a boy resulting from a pregnancy with placental steroid sulphatase C deficiency who did not develop an ichthyotic condition.

Investigations

We investigated histochemically the presence of arylsulphatase activity instead of steroid sulphatase because no histochemical method is known for the latter. We investigated the skin of five of these boys (Cases 1, 2, 3, 4 and 5), of eighteen adults with sex-linked ichthyosis, of the mothers of three of the boys, of two other carriers, of fifteen healthy controls and of a number of patients with various other keratotic conditions (Table 1). Strong arylsulphatase C activity was present in the granular layer of the epidermis of fifteen healthy controls but was absent in this layer in the five boys and in fourteen adults with sex-linked ichthyosis; in the other four a very slight activity was present. The five carriers failed to show enzyme activity. This last rather unexpected result is probably due to the failure of histochemical techniques to demonstrate low levels of activity such as may be expected in carriers. In the other histochemically investigated conditions arylsulphatase C activity was demonstrated in the skin of all investigated patients with psoriasis, seborrhoeic warts, actinic keratoses and in one case of Netherton's syndrome. We found no activity in eleven of thirteen cases of dominant ichthyosis.

Skin fibroblasts of Cases 2, 3 and 6 were grown in culture and tested for steroid sulphatase C activity. The values found in these cases were 10–40 times lower than in seven normal controls.

TABLE 1. Results of histochemical investigation of the skin for arylsulphatase C activity in healthy controls, patients with sex-linked ichthyosis and a variety of other disorders

	ASC positive*	ASC negative†
Healthy controls (15)	15	0
Cases 1–5	0	5
Sex-linked ichthyosis (18)		
Hemizygotes	4(±)	14
Heterozygotes	0	5
Psoriasis (10)	10	0
Actinic keratoses (3)	3	0
Seborrhoeic warts (3)	3	0
Autosomal dominant ichthyosis (13)	2	11
Netherton's syndrome (1)	1	0

*Arylsulphatase C present in granular layer.
†Arylsulphatase C absent in granular layer.
±Positive, but very slight.

Investigation of the placenta of five of our cases showed absence of arylsulphatase C histochemically and extremely low values for steroid sulphatase C biochemically (Table 2).

Shapiro *et al.* (1978a, b) who in consequence of our publications (Jöbsis *et al.*, 1976; Koppe *et al.*, 1977) were the first to measure steroid sulphatase activity in cultured skin fibroblasts found an almost totally absent activity in all cases of sex-linked ichthyosis. Kubilus, Tarascio & Baden (1979) confirmed

TABLE 2. Results of histochemical and biochemical investigation

	Maternal urine	Skin				Placenta	
Case no.	Oestrogen (μmol/24h)	Ichthyosis S-L	Ichthyosis S-L?	Epid. ASC	Fibrobl. SS (pmol/h, mg protein)	Trophobl. ASC	Homogenate SS (nmol. DHEA-S/h. mg protein)
1	<20	+		—	n.d.	—	<0·3
2	<20	+		—	10	—	<0·3
3	<18		+	—	15	—	<0·4
4	<20	+		—	N.D.	N.D.	N.D.
5	<15	+		—	N.D.	—	<0·4
6	<25		+	N.D.	15	N.D.	<0·2
Normal	32–179	—	—	+	200–765	+	12–48

Ichthyosis S-L: sex-linked; S-L?: probably sex-linked. Epid. ASC: presence of arylsulphatase C in granular layer, histochemical.

Fibrobl. SS: steroid sulphatase C in cultured fibroblasts, biochemical.

Trophobl. ASC: presence of arylsulphatase in trophoblast, histochemical.

Homogenate SS: steroid sulphatase in homogenate of placenta, biochemical.

DHEA-S: dehydroepiandrosterone sulphate.

N.D.: not done.

this, and moreover found no enzyme activity in cultured epidermal cells. Normal values were found in four patients with ichthyosis vulgaris (Shapiro *et al.*, 1978a; Kubilus, *et al.* 1979).

Recently Shapiro, Mohardas & Weiss (1979) concluded from their work on fibroblasts cultured from four female obligate heterozygotes that the region of the sulphatase locus on the X-chromosome is not subject to lyonization. This is an interesting observation; however it is then difficult to explain how some carriers show ichthyosis, albeit in a very slight degree, on their extremities.

DISCUSSION

In six cases of proven placental sulphatase deficiency boys were born presenting with ichthyosis soon after birth. In all of them sex-linked ichthyosis was more likely than dominant ichthyosis. The assumption that the combination of placental sulphatase deficiency and ichthyosis is coincidental is unlikely because:

(a) both conditions are probably sex-linked;

(b) both are relatively rare;

(c) as yet we have not come upon a boy from a pregnancy with steroid sulphatase deficiency who did not develop ichthyosis. It would be important to investigate the urine of all pregnant women in a family known with sex-linked ichthyosis. We have not yet had the opportunity to do this. We believe, that sulphatase deficiency is of major importance in the pathophysiology of sex-linked ichthyosis because:

(a) arylsulphatase C activity was absent or, in a few cases, very feeble in the granular layer of the epidermis of all investigated adults with sex-linked ichthyosis as well as in the five boys we could investigate histochemically;

(b) steroid-sulphatase activity was virtually absent in the fibroblasts of three boys from whom we could culture these cells, including the one in whom no histochemical investigation had been performed. This result is in accordance with the work of Shapiro *et al.* (1978a, b) and Kubilus *et al.* (1979) on sex-linked ichthyosis;

(c) a minor argument might be the presence of ichthyosis in multiple sulphatase deficiency as reported in the literature (Rampini *et al.*, 1970; Austin, 1973).

The question as to how arylsulphatase C, steroid sulphatase C or both enzymes are involved in the keratinization process is still open. Hameister *et al.* (1979) suggested as an alternative possibility in placental steroid sulphatase deficiency another primary defect causing a reduction of several enzyme activities. This hypothetical third factor could also have an effect on keratinization.

We are investigating the cause of the negative histochemical results for arylsulphatase C activity in dominant ichthyosis. Unreliability of the histochemical method does not seem to be the explanation, because not only in healthy controls but also in various other keratotic conditions arylsulphatase activity could always be demonstrated by this method. Obviously the histochemical method cannot be used to distinguish between sex-linked and dominant ichthyosis. Biochemical investigation of cultured cells however will probably prove to be a reliable tool.

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British Journal of Dermatology (1980) 103, 81.

Case Reports

Anderson–Fabry disease

CHARACTERISTIC ULTRASTRUCTURAL FEATURES IN CUTANEOUS BLOOD VESSELS IN A 1-YEAR-OLD BOY

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SUMMARY

Electron microscopy of clinically uninvolved skin taken from a 12-month-old male child with biochemically proven angiokeratoma corporis diffusum showed characteristic lamellar lipid deposits within endothelial and perithelial cells of dermal blood vessels. Ultrastructural examination of skin may aid the early identification of males affected with Anderson–Fabry disease long before the development of specific skin lesions.

Angiokeratoma corporis diffusum (Anderson–Fabry disease) is a sex-linked disorder of sphingolipid metabolism (Sweeley & Klionsky, 1963) in which a deficiency of the enzyme ceramide trihexosidase (Brady *et al.*, 1967) leads to the abnormal deposition of glycolipid throughout the body. Renal damage, and cerebrovascular and cardiac disturbances are common and the average life expectancy of affected males is only 42 years (Wallace, 1973). The cutaneous angiectases typical of this condition are uncommon before puberty and seldom conspicuous before the late teens (de Groot, 1970; Wallace, 1973). We describe the occurrence of angiokeratoma corporis diffusum in the great-great-grandson of the patient originally reported by Anderson (1898), and demonstrate that ultrastructural abnormalities in the skin are detectable within the first year of life.

CASE REPORT

A 12-month-old male child was admitted for assessment. The patient was the great-great-grandson of Anderson's original patient with angiokeratoma (Anderson, 1898) and the grandson of the male patient designated Case 19 in the series reported by Wise, Wallace & Jellinek (1962). His mother, aged 27, was known to have a corneal dystrophy and subnormal leukocyte α-galactosidase activity. An elder sister, aged 4, was clinically normal but not examined biochemically.

On examination the patient appeared to be a normal child without any ocular or cutaneous lesions. Biochemical investigation revealed a low leukocyte α-galactosidase activity at 6 nmol substrate/mg

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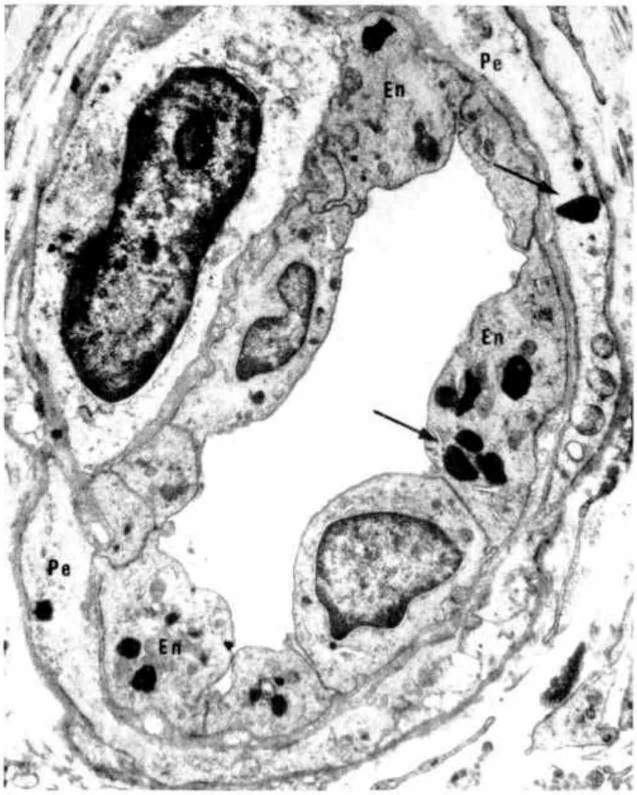

FIGURE 1. Transverse section of a dermal vessel showing endothelial cells (En) and perithelial cells (Pe) containing cytoplasmic inclusion bodies (arrowed) (× 5000).

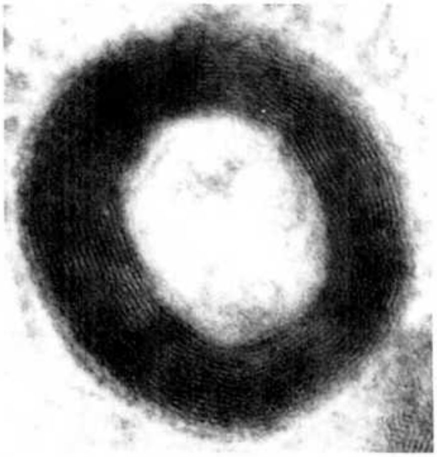

FIGURE 2. High power view to show internal lamellar structure of inclusion bodies (× 125,000).

protein/h (normal 40–120) and a low plasma α-galactosidase activity at 0·5 nmol substrate/ml/h (normal 4–12). The leukocyte β-galactosidase activity was normal.

A skin biopsy examined under the electron microscope revealed the presence of electron-dense inclusion bodies within the cytoplasm of endothelial and perithelial cells (Fig. 1), and at high resolution these bodies were seen to have a characteristic lamellar ultrastructure (Fig. 2).

DISCUSSION

On light microscopy the skin lesions in angiokeratoma corporis diffusum consist of dilated blood-filled vessels in the upper dermis lying beneath a thinned epidermis with or without hyperkeratosis. The amount of lipid in the skin in Anderson–Fabry disease is too small to be detected without the use of special fixation and lipid-staining techniques. The ultrastructural changes are, by contrast, striking and the diagnosis may be made more easily with the electron than the light microscope (Schatzki, Kipreos & Payne, 1979). Electron microscopic changes in the skin in Anderson–Fabry disease have been reviewed by Frost, Tanaka & Spaeth (1966), Sagebiel & Parker (1968), Perrelet *et al.* (1969), Van Mullem & Ruiter (1970) and Hashimoto, Lieberman & Lamkin (1976). Electron-dense cytoplasmic inclusion bodies with a lamellar internal organization, consisting of alternating electron opaque and light regions with a periodicity of 40–60 Å, are found in endothelial and muscle cells of blood vessels, perithelial and perineural cells, arrectores pilorum muscles and dermal macrophages, and it has been suggested that they represent lipid deposits within lysosomes (Van Mullem & Ruiter, 1970; Hashimoto *et al.*, 1976). Inclusion bodies have been reported in the skin of adult patients even in the absence of clinically evident angiectases (Clarke *et al.*, 1971; Volk *et al.*, 1974), and de Groot (1970) found lipid material at the light microscope level in the walls of dermal vessels in apparently normal skin from a girl aged 5 and a boy aged 6 years. Electron microscopy of the skin in our patient revealed that the characteristic ultrastructural changes are present within the first year of life.

In recent years attention has been focused on the possibility of enzyme replacement therapy in Anderson–Fabry disease (Krivit *et al.*, 1972; Brady *et al.*, 1973). The finding of a tenfold increase in the level of ceramide trihexoside in the liver of a 21-week-old affected male fetus (Brady, Uhlendorf & Jacobson, 1971) emphasizes that early treatment will be necessary to prevent the long-term consequences of continued lipid deposition. Electron microscopy of skin provides an additional diagnostic technique based on morphological rather than biochemical criteria in the identification of presymptomatic cases.

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Generalized trichorrhexis nodosa

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SUMMARY

A case of generalized trichorrhexis nodosa is described in a man. Electron histochemical studies showed abnormal staining consistent with a definite disorder in the formation of alpha-keratin chains within the globular matrix of the hair cortex with respect to cystine; keratin amino-acid analysis also revealed low cystine. This cortical change, together with multiple vacuoles found in the endocuticle, are evidently the intrinsic defects which allow relatively trivial chemical and physical trauma to cause trichorrhexis nodosa.

Trichorrhexis nodosa of the scalp is a common disorder of adult women as a consequence of excessive cosmetic treatment (Rook, 1976); however, such cases are rarely referred for dermatological advice. Generalized trichorrhexis nodosa of unknown cause in adults is rare. We here report such a case and present investigations to suggest a possible pathogenesis.

CASE REPORT

A 45-year-old Caucasian presented with a 5-month history of white flecking and abnormal breakage of his hair. He admitted to regular vigorous massage of his scalp but not to any other significant physical or chemical insult. He was otherwise in good health. There was no family history of ectodermal abnormality.

On examination white nodes typical of trichorrhexis nodosa were seen on the hair shafts. The nodes were sometimes multiple. They were visible on the scalp and other sites, most noticeably the thighs and backs of his hands. His skin was otherwise normal.

Investigations

Light microscopy

Light microscopy confirmed the presence of nodes and showed cuticular disruption independent of them. Cuticular disruption was also seen on the hairs that appeared clinically normal (Fig. 1).

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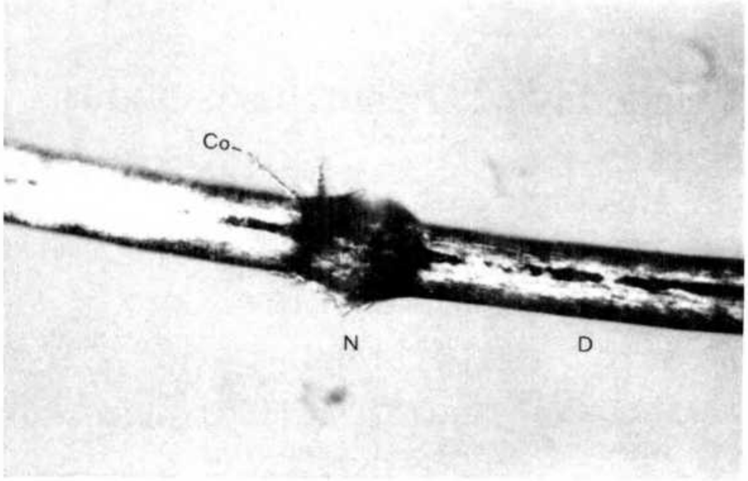

FIGURE 1. Abnormal hairs from back of hand seen with plane polarised light showing node of trichorrhexis nodosa (N), splayed cortical cells (Co) and cuticular disruption (D).

Electron microscopy

(*i*) *Method.* Samples of affected hair shafts were embedded in Spurr's low viscosity resin (Spurr, 1969) and sectioned on an LKB (III) microtome. Transverse sections of 500 Å thickness were transferred to Formvar coated gold grids for viewing with a Siemens Elmistop 101 transmission electron microscope. The sections were stained by the following techniques: (i) uranyl acetate and lead citrate—general stain; (ii) dodeca-tungstophosphoric acid—cortex matrix proteins and cortical cell membranes; (iii) silver methenamine cystine specific stain (Swift 1968). No attempt was made to clear debris from the hair samples because of the unstable nature of the hair shaft in trichorrhexis nodosa. Sections were cut at both nodal and internodal sites.

(*ii*) *Results.* The overall transverse structure was circular to slightly oval. All the sections showed numerous pigment granules. At both nodal and internodal sites the hair showed varying degrees of cuticular loss; at some points the cortex was exposed due to total loss of cuticle.

The cuticle cells remaining on the shaft showed a normal complement of laminations. Numerous small vacuoles were seen in the endocuticle (Fig. 2). In places these coalesced resulting in complete splitting of the endocuticle. Radial breakage of the cuticle cells appeared to be the result of weathering and not due to a weakness in cuticle cell function. Where total cuticle loss had occurred cortical cells were directly exposed and tended to show degenerative change.

Splitting of the cortex occurred throughout its mass, the breaks following cortical cell boundaries although the membranes did not appear abnormal. Within the bulk of the cortex the cells appeared to be intact; however, at the periphery some cells were split into individual macrofibrils giving the splayed effect seen at the node. With specific staining for cystine many of the cortical cells showed typical fingerprint whorls (Fig. 3). Others showed a random arrangement of cystine residues (Fig. 4a) with an increased distance between adjacent cystine-positive macrofibrils compared with normal hair (Fig. 4b).

Breaking stress

Quantitative breaking stress analysis was estimated using a Cambridge stress tester (Table 1). The

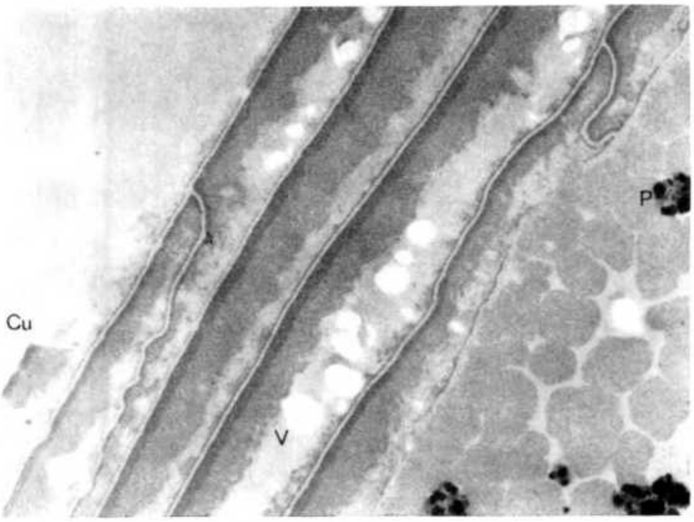

FIGURE 2. Transverse section illustrating vacuole inclusions (V) within the endocuticle. Cuticle cell debris (Cu) is visible at the periphery.

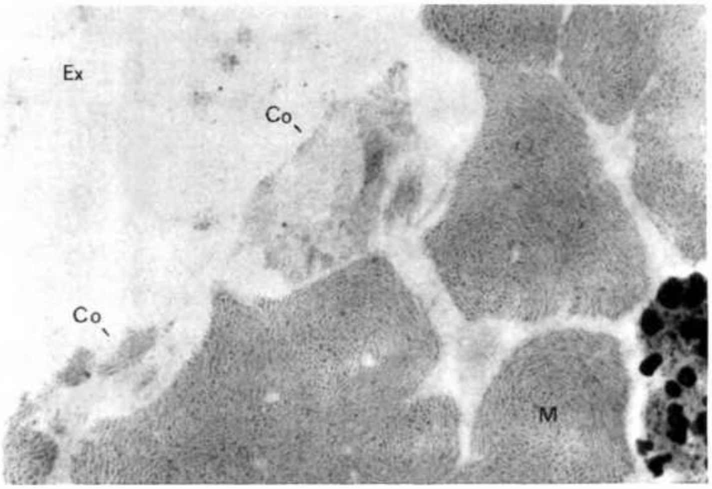

FIGURE 3. Transverse section illustrating total cuticle loss and degenerative changes in peripheral cortical structures (Co). Undamaged macrofibrils (M) are still visible. Ex = external.

results show that the clinically normal hairs (free from nodes though showing cuticular disruption microscopically) were weaker than normal controls but not so weak as those with nodes. Microscopic examination of fractured hair shafts possessing nodes showed breakage to occur through a node.

Amino acid analysis of the hair

Amino acid analysis of the hair showed the cystine content to be significantly lower than in normal controls (Table 2).

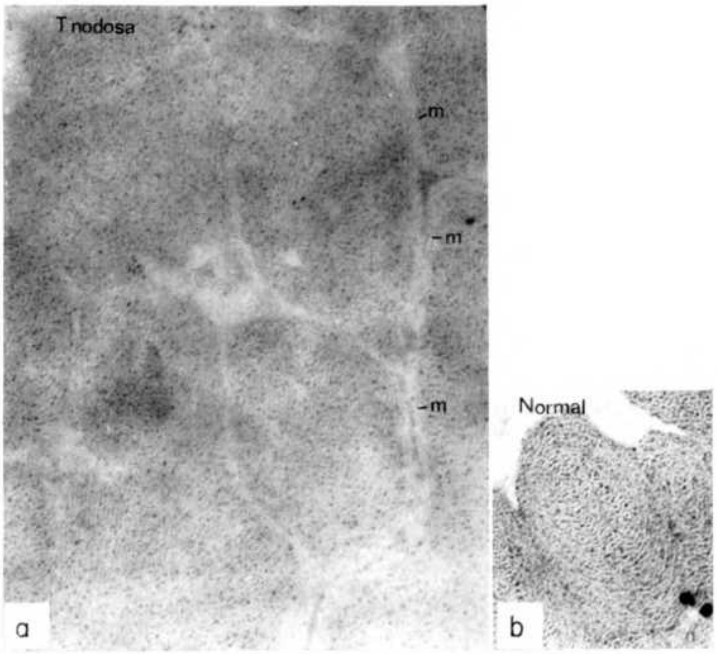

FIGURE 4. (a) Transverse section of cortex of trichorrhexis nodosa illustrating irregular microfibrillar staining. (b) Comparative transverse section of normal hair. Both sections stained with silver metheramine. m = Cortical cell membrane.

TABLE 1. Hair breaking stress analysis

		Patient's hair	
	Normal control	With nodes	Without nodes
Mean breaking stress (kg mm^{-2})	20·3	7·4	14·1
No. of tests	40	40	40

Urinary amino-acid chromatography
Normal.

DISCUSSION

Trichorrhexis nodosa may be the consequence of hereditary diseases e.g. amino-acid disorders (Shelley & Rawnsley, 1965; Raushkolb *et al.*, 1967) and deformed hair shaft disease, e.g. pili torti (Dawber, 1977). The former group may present late in life and thus amino acid chromatography is obligatory in all cases.

For normal hair to acquire trichorrhexis nodosa it must be subjected to severe physical or chemical trauma as with perming or bleaching (Chernosky, 1974). The cuticular cells then become disrupted allowing the cortical cells to splay out leading to node formation (Dawber & Comaish, 1970).

TABLE 2. Amino acid composition of hair shafts expressed as a percentage of total nitrogen (excluding ammonia) recovered from the column (Pollitt & Stonier, 1971).

Amino acid	Normal mean ± s.d.	Patient
ASP	4·3 ± 0·2	4·5
THRE	6·0 ± 0·2	6·2
SER	9·6 ± 0·3	9·7
GLU	9·7 ± 0·2	10·1
PRO	7·3 ± 0·3	7·1
GLY	5·1 ± 0·4	4·9
ALA	3·8 ± 0·1	3·9
CYST	14·4 ± 0·4	9·7
VAL	4·4 ± 0·1	4·5
MET	0·4 ± 0·1	0·4
I-LEU	2·2 ± 0·1	2·3
LEU	5·1 ± 0·1	5·1
TYR	1·7 ± 0·1	1·7
PHE	1·4 ± 0·1	1·3
HIST	2·1 ± 0·2	2·2
LYS	4·3 ± 0·4	4·2
ARG	18·3 ± 0·6	18·3

This case is that of a man with no evident hereditary disorder to account for the presence of generalized trichorrhexis nodosa. It is unrealistic to suggest that massage alone could account for such widespread involvement of hair.

The staining variations seen on electron microscopy could not be accounted for by para- and orthocortical cell differences, nor were they an artefact of preparation. Overall consideration of the cortex indicates a definite disorder in the formation of alpha protein chains within the globular matrix of the cortex with respect to cystine. The differential staining encountered in the cortex is indicative of abnormal cystine incorporation—the degree of this could be tested by selective amino acid extraction (Gillespie, 1963).

The endocuticle represents the cellular remnants of the cuticle cells and consequently is not crosslinked by disulphide bonds. The inclusion of vacuoles therefore results in structural weakness. Actual splitting of the endocuticle is then induced by physical and chemical weathering. Similar vacuoles are seen in normal hair but to a lesser extent and do not appear to affect the hair except at the distal ends of long hairs which would have been subject to prolonged weathering, unlike the short hairs found in trichorrhexis nodosa.

The finding of a low cystine level in this patient's hair is consistent with the electron microscope findings. The disulphide bonds formed by this amino acid are known to be important in giving normal keratin its great strength (Spearman, 1977). Cases of trichorrhexis nodosa have been described in two mentally retarded siblings with amino-succinic aciduria, the hairs from which were deficient in cystine (Pollitt, Jenner & Davies, 1968).

We suggest that the patient described here has an idiosyncratic defect in hair keratin synthesis that makes it prone to damage by trivial trauma. The fact that several nodes occurred on a single short shaft indicates that they are the result of dynamic forces acting on the shaft with the points of flexion being at the skin surface as the shaft emerges from the pilary canal. It is interesting to speculate that a similar abnormality of keratin synthesis might have accounted for previous reports of partial alopecia due to massage (Bowers, 1950).

ACKNOWLEDGMENTS

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Infection of the finger-nail by *Pyrenochaeta unguis-hominis*

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SUMMARY

Infection of the finger-nail of an elderly woman by *Pyrenochaeta unguis-hominis* is reported. The small lesion was extending slowly into the healthy nail plate. The fungus has been isolated from diseased toe-nails on two previous occasions but its extra-human habitat is unknown.

Pyrenochaeta unguis-hominis Punithalingam and English was first described in 1975. It had been isolated from the toe-nails of an elderly woman and was identical with a *Pyrenochaeta* sp. which had been obtained from the discoloured toe-nails of an elderly man by English & Atkinson (1974). Both patients came from the West of England and in both cases hyphae could be seen on direct microscopy of the nail. The fungus had never been reported from any other substrate and there have been no further records of it since 1975, until the culture from the present case was briefly mentioned by Punithalingam (1979).

CASE REPORT

In August 1977 an 85-year-old woman living in the East Midlands complained of a small area of brownish discolouration on her R5 fingernail (Fig. 1a). The lesion was increasing in size very slowly. The patient had suffered from arthritis for 20 years and her domestic activities included light washing, washing-up and the preparation of food for cooking. A clipping from the nail was sent to Bristol, but no fungus was found on direct microscopy and only small quantities of *Candida albicans* and *Staphylococcus aureus* were isolated in culture. The nail was treated with Brulidine cream.

In June 1978 the patient was visited in her home. She said that the discoloured area had extended very slightly during the previous year. The edge of the lesion was sharply demarcated and the rest of the nail plate appeared to be healthy, as did her other finger-nails with the exception of the L4 nail (Fig. 1b), but this lesion was so small that it was impossible to obtain an adequate clipping from it. Clippings were obtained from deeper into the R5 lesion than those taken previously, and on microscopic examination dense fungal mycelium was seen. Cultures yielded *P. unguis-hominis* but no *Staph. aureus* or *C. albicans*. Similar results were obtained from further clippings taken in April 1979. In July 1979 the patient died.

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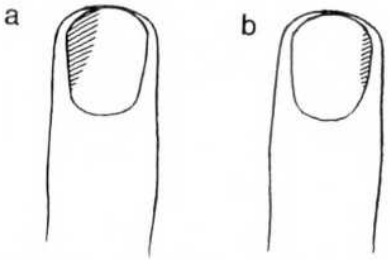

FIGURE 1. Lesions of finger-nails. (a) R5 nail; (b) L4 nail.

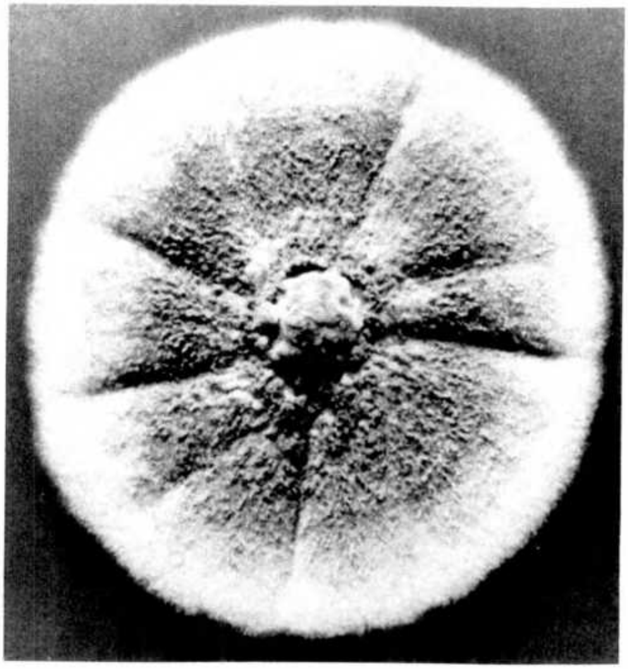

FIGURE 2. Colony of *Pyrenochaeta unguis-hominis* (× 2).

Colonies of *P. unguis-hominis* are slow-growing, and dark grey-green in colour with a broad, whitish border. They are heaped in the centre, with deep, radial folds and the minute tufts of aerial mycelium with which they are covered give the surface a rough appearance (Fig. 2). The fungus is difficult to identify as sporulation does not occur under ordinary laboratory conditions, though it can be induced by submitting cultures to alternating periods of 'black light' (near-ultra violet irradiation) and darkness. *P. unguis-hominis* is a pycnidial fungus, that is, the sporogenous apparatus and spores develop within dark-coloured, thick-walled, flask shaped structures known as pycnidia, from which they are extruded at maturity.

DISCUSSION

This case has established that *P. unguis-hominis* grows actively in the nails for the discoloured area was extending, though slowly, into the healthy nail plate after *C. albicans* and *Staph. aureus* had been eliminated. Whether it is a primary or a secondary pathogen is less clear. *C. albicans* and *Staph. aureus*, though found in only very small quantities, might have been responsible for initiating the lesion, which was then invaded by *P. unguis-hominis*. However, the first clippings examined were not very adequate and *P. unguis-hominis* could have been present from the first, while the other two organisms were chance contaminants.

P. unguis-hominis has now been isolated twice from the toe-nails and once from the finger-nails of elderly persons, each time by the same worker, but it has still not been reported from any other source. Yet in reality it is almost certainly, like other moulds infecting nails, a saprobic fungus, probably a member of the soil mycoflora, that can also colonize nails already predisposed to infection by age, trauma or disease. Further reports of its occurrence on or off the human body would be of considerable interest.

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Cutaneous cryptococcosis

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SUMMARY

A 31-year-old woman with long-standing renal disease, treated with systemic steroids and azathioprine, developed progressive skin ulceration and subcutaneous nodules. A diagnosis of cryptococcosis was established after histological examination of a cutaneous lesion and confirmed by culture of the organism from the biopsy specimen. A detailed description of the histology and ultrastructure of the cutaneous lesion is presented. Treatment with parenteral amphotericin B and 5-fluorocytosine resulted in dramatic resolution of the skin lesions.

Cryptococcosis is an opportunistic infection caused by the encapsulated yeast *Cryptococcus neoformans*. The usual portal of entry in humans is the respiratory tract from which there is haematogenous spread to internal organs, primarily the meninges. Only 10% of patients with cryptococcosis develop cutaneous lesions. These may present as multiple small papules, nodules, non-healing ulcers (Rook & Woods, 1962), ecchymoses (Cawley, Grekin & Curtis, 1950) acneform lesions, granulomas, gummatous lesions, subcutaneous abscesses or tumour-like swellings, with or without sinus formation (Moore, 1957). They may also mimic bacterial cellulitis (Gauder, 1977). Our patient demonstrated some of the typical skin lesions of this condition, and histology of these illustrated the full range of tissue reaction seen in this infection.

CASE HISTORY

A 31-year-old Maltese female developed acute glomerulonephritis in 1969, succeeded by the nephrotic syndrome. Investigations for suspected underlying systemic lupus erythematosus were negative, but treatment comprising a combination of azathioprine and dexamethasone was instituted. Subsequent renal biopsy in 1970 revealed membrano-proliferative glomerulonephritis with features suggestive of lupus nephropathy. However, her renal function improved and she was weaned off treatment, whereupon she remained well until 1974. Then she developed diarrhoea and vomiting followed by a gradual deterioration in her renal function. Treatment with azathioprine and dexamethasone was reinstituted.

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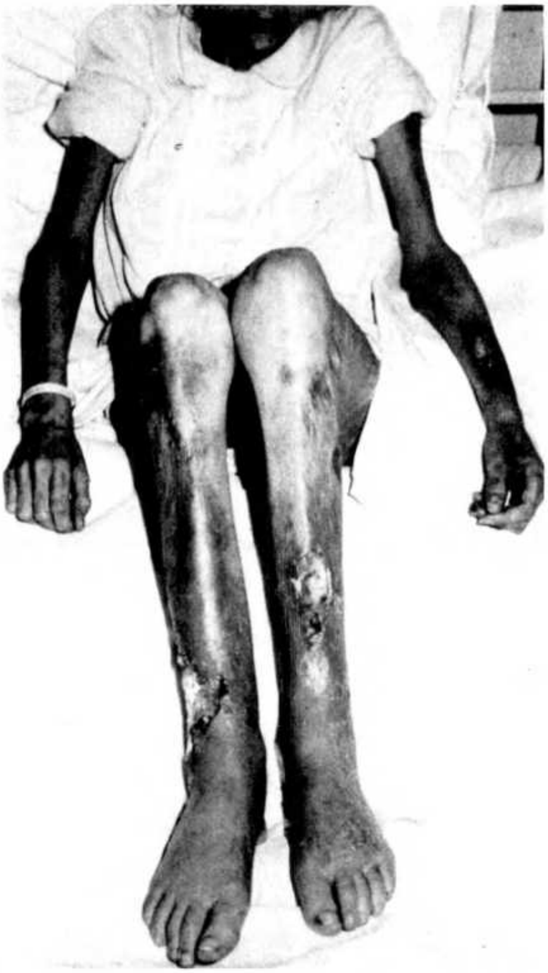

FIGURE 1. Cutaneous cryptococcosis. Ulcerated and nodular lesions on the legs and left arm.

Very high doses of steroids were required to control her renal disease, and because of this she subsequently developed steroid cataracts, myopathy and oesophagitis. In January 1978, she developed an abscess on her left buttock which was incised and healed rapidly. Later that year she developed an ulcer on her left wrist which gradually extended despite systemic antibiotics and topical antiseptics. Subsequently there was progressive ulceration of both her lower legs and left thigh. At the time of referral to the dermatology department, deep secondary infected ulcers were present on the lower aspects of both legs, on her left thigh and left wrist and a large nodule was noticed on the left forearm (Fig. 1). The skin around the ulcers appeared red and oedematous resembling cellulitis. She appeared cachectic with extensive pitting oedema of the legs and sacrum and she had a low grade pyrexia. There was no lymphadenopathy.

Investigations

She was anaemic (Hb 8·6 g/l) with a mild mainly polymorphonuclear leukocytosis. Tests for antinuclear factor and DNA binding were negative. Renal biopsy at this stage showed a picture similar to that

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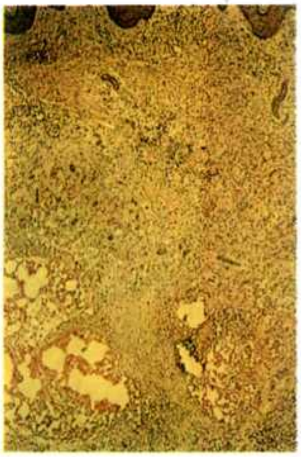

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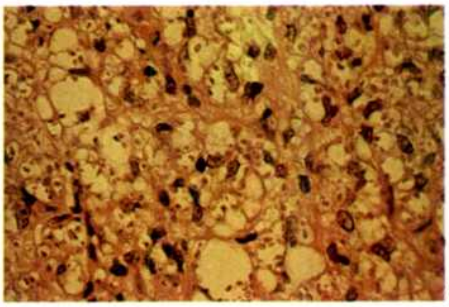

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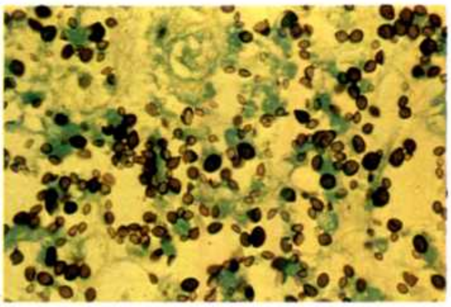

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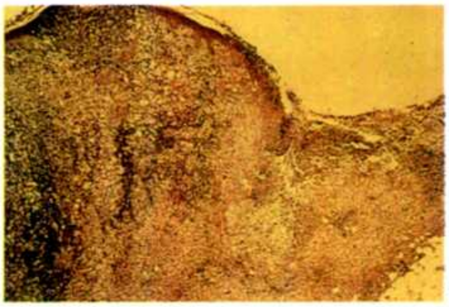

FIGURE 2. Cutaneous cryptococcosis. A mixed chronic inflammatory infiltrate permeates the entire depth of the dermis. In the mid dermis there is a prominent area of granulomatous infiltrate incorporating numerous giant cells. Dense collections of cryptococci forming 'toruloma'-like areas are situated deeply (H & E, × 60).

FIGURE 3. Cutaneous cryptococcosis. Numerous yeast-like organisms are surrounded by clear halos representing gelatinous capsules (H & E, × 125).

FIGURE 4. Cutaneous cryptococcosis. Individual yeasts show budding and pseudohyphae formation (Grocott silver stain, × 300).

FIGURE 5. Cutaneous cryptococcosis. An ulcerated lesion showing a subepidermal neutrophil polymorph abscess in the ulcer margin. Chronic inflammatory infiltrate incorporating prominent collections of lymphocytes extends into the deep dermis (H & E, × 25).

of 1970 with membranoproliferative glomerulonephritis. The diagnosis of cryptococcosis was made histologically on biopsies of the cutaneous nodule and an ulcerated lesion and by the isolation of *Cryptococcus neoformans* from the biopsy material. Subsequent lumbar puncture showed the presence in the cerebrospinal fluid of 20 leukocytes, 80% of which were neutrophils. On further questioning, she described intermittent headaches for approximately 2 years. Cryptococcal antigen in a titre of 1:25 was present in the cerebrospinal fluid indicating meningeal involvement, but was found in the blood on only one occasion in undiluted serum.

Histopathology

Nodular and ulcerative lesions were biopsied for histological examination. In haematoxylin and eosin (H & E) sections of the nodular lesion an area of tissue necrosis incorporating degenerating cells and pyknotic nuclei was present in the reticular dermis. Peripheral to this, isolated areas of a granulomatous inflammatory infiltrate, including many histiocytes and giant cells and smaller numbers of plasma cells and lymphocytes, were randomly distributed. Between these areas of cellular reaction in the dermis, variable sized zones of mucoid change were seen in the dermis (Fig. 2). At high magnification innumerable small yeast-like organisms were seen to fill these 'gelatinous' areas and were also seen scattered amongst the inflammatory infiltrate and within histiocytes and giant cells. Individual yeast-like bodies were surrounded by a clear halo of varying size suggestive of a mucinous capsule (Fig. 3). The Grocott silver stain demonstrated the variation in size of the individual yeasts which frequently showed the formation of single, multiple or chains of buds in the tissue (Fig. 4). The distribution of organisms was seen to be much more widespread than was apparent in the H & E stained sections and extended from the papillary dermis into the subcutaneous fat. The yeast capsules stained with mucicarmine displaying a red colour which was most evident in the 'gelatinous' zones. Here the picture was reminiscent of a 'toruloma', a focal zone containing many organisms and minimal inflammatory response, which may arise in cerebral tissue in systemic cryptococcosis. The size of the capsule surrounding phagocytosed organisms was considerably attenuated. The only change visible in the epidermis was mild acanthosis.

By contrast, in the second specimen there was extensive ulceration of the epidermis with underlying granulation tissue. No discrete areas of frank tissue necrosis were visible, but there was a prominent mixed granulomatous cellular reaction and areas of 'toruloma'-like change similar to those seen in the first specimen. In addition lying in the papillary dermis below non-ulcerated epidermis, a large neutrophil polymorph abscess incorporating nuclear remnants and a few degenerating organisms was present (Fig. 5).

Electron microscopy

Numerous intracellular yeasts were present within the cytoplasm of host macrophages and in some of these budding could be seen. In many cryptococcal cells there was considerable intracellular disorganization. The fibrillar remnant of the capsule could be distinguished on the periphery of some yeasts. The cell wall was represented by a zone of medium to high electron density external to the plasma membrane. A clear zone lay between capsular remnants and cell wall (Fig. 6). In one cell there was an unusual cytoplasmic organelle consisting of a condensation of concentrically arranged membranes. This was similar in appearance to the bacterial mesosome and has been seen in cryptococci grown *in vitro* (Edwards *et al.*, 1967).

Subsequent course

Initially the patient was treated with intravenous miconazole for 3 weeks, and the dose was increased from 100 to 1000 mg three times daily. A total of 48·2 g was given and in the last week oral 5-fluoro-

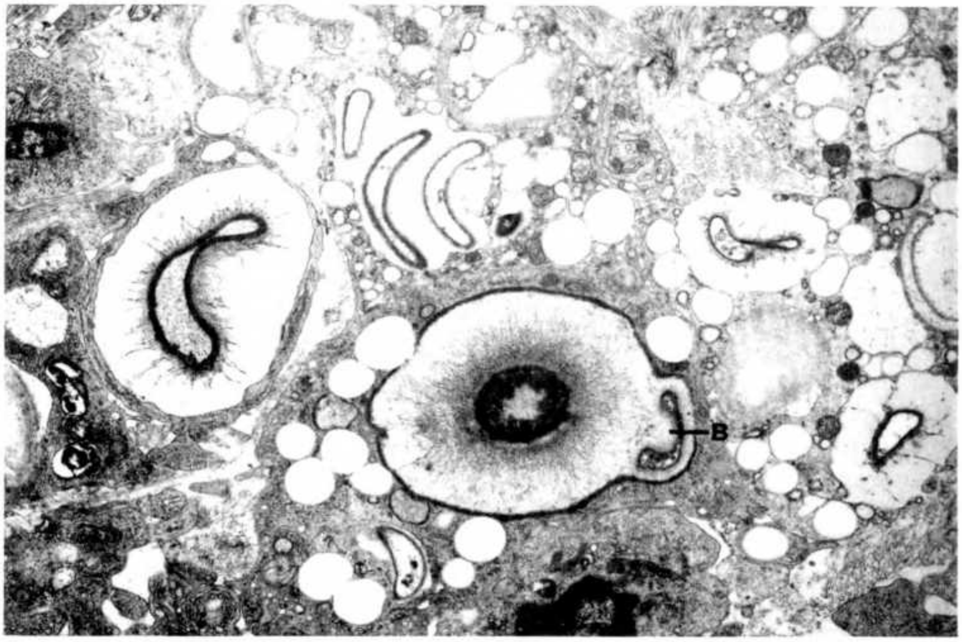

FIGURE 6. Cutaneous cryptococcosis. Macrophages containing cryptocci in various stages of lysis. In the foreground there is a budding yeast which is surrounded by fibrillar material representing the capsule. B = budding yeast (× 5500).

cytosine was added to the regime. Despite this she remained pyrexial and there was no clinical improvement in the skin lesions. At this stage the treatment was changed to a combination of intravenous amphotericin B and oral 5-fluorocytosine which was administered in two courses separated by an interval of 4 weeks. The dose of 5-fluorocytosine was 2 g twice daily and this was sufficient to maintain her blood level in the range of 60–70 mg/100 ml. The total dose of amphotericin B given was 2·3 g. In addition, at the start of this course of treatment, amphotericin B was given intrathecally twice weekly to a total dose of 1·8 mg. On this treatment there was rapid resolution of her skin lesions. Despite a mild relapse when the therapy was stopped for 4 weeks, her skin lesions were clinically healed at the end of the course of treatment and there was no histological evidence of persistent infection.

DISCUSSION

The clinical progress of cases of cryptococcosis with accompanying cutaneous lesions is very variable, ranging from indolent forms (Rook & Woods, 1962) to those with a rapidly downhill course (Gauder, 1977). In particular, Nottebart, McGehee & Utz (1973) suggested that cryptococcosis in patients with sarcoidosis evolves more slowly. It is difficult to be certain about the duration of the illness in our patient although cutaneous ulceration was recorded over a year before the diagnosis was established. The morphology of her lesions included nodules, abscesses, ulcers and cellulitis. The cellulitis was particularly prominent during a relapse when treatment was interrupted but rapidly resolved on reinstitution of amphotericin B and 5-fluorocytosine.

Normally, direct inoculation of systemic pathogenic fungi such as *Histoplasma* into the skin results in a characteristic syndrome, the cutaneous chancriform syndrome (Wilson, 1963). The process is usually self-limiting and dissemination has been seen only in coccidiodmycosis (Wright, Newcomer & Nelson, 1959). Although primary cryptococcosis can be established by direct inoculation of organisms into animals, e.g. mice (Dykstra & Friedman, 1964), on the rare occasions where cryptococci have been inoculated into human skin (Halde, 1978) no local or systemic infection has occurred. Even in reported cases of 'primary cutaneous cryptococcosis', proof of infection by cutaneous inoculation has never been established and in many of these instances there was eventual dissemination of the disease (Noble & Fajardo, 1972). Hence it is difficult to determine whether a cutaneous lesion follows local inoculation or arises as a result of focal metastatic spread from the lung.

Although the skin lesions in our case were the predominant presenting feature, the co-existence of an underlying meningitis emphasizes the need to investigate all such patients for systemic involvement.

The histological features of cryptococcosis are very variable and range from dense tuberculoid granulomata with few organisms to large clusters of yeasts without significant reaction (Baker & Haugen, 1955). More rarely polymorphonuclear leukocyte abscesses may be present (Symmers, 1953). Our patient demonstrated all of these histological features.

In addition the capsule was readily demonstrable by the mucicarmine stain and ultrastructurally showed the fibrillar structure previously reported (Edwards *et al.*, 1967; Noble & Fajardo, 1972). Occasionally unencapsulated or weakly encapsulated cryptococci (the dry variant) may be clustered within histiocytes closely resembling *Histoplasma capsulatum* in shape and size (Gutierrez, Fu & Lurie, 1975). This was not observed in our patient.

The results of a combined regime of 5-fluorocytosine and amphotericin B in cryptococcosis have recently been recorded (Bennett *et al.*, 1979) and the authors report a low frequency of relapse, rapid sterilization of lesions and an improved cure rate. Our patient responded well to the regime although 5-fluorocytosine serum levels had to be monitored closely and adjusted if necessary to avoid toxic reactions, e.g. granulocytopenia. In addition a large dose of amphotericin B (over 2·3 g *in toto*) was required to achieve resolution in our patient. There are no established criteria governing length of treatment and complete success of therapy must be determined by factors such as clinical healing and histological and cultural evidence of death and disappearance of the organisms from the tissue. Although miconazole has been shown to be effective in cryptococcosis (Graybill & Levine, 1978) there was no response in our patient to this drug either by itself or in combination with 5-fluorocytosine. More data are obviously needed on the use of miconazole in human cryptococcosis.

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Brief Communications

Heterozygote detection in Fabry's disease using mailed hair roots

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Hair roots can successfully be employed as an easily obtainable biopsy material for heterozygote detection in inborn errors of metabolism (Gartler *et al.*, 1971; de Bruyn, Oei & ter Haar, 1974; De Bruyn *et al.*, 1979).

We have recently described an easy and rapid method for measuring α-galactosidase and acid phosphatase activities in individual human hair roots (Vermorken *et al.*, 1978). It was shown that acid phosphatase is a suitable reference enzyme with respect to α-galactosidase since it has a comparable distribution in the hair follicle and it is equally stable on storage. In this study we report that mailing hair roots does not interfere with the possibility of measuring the enzyme activities and distinguishing between patients, heterozygotes and normal controls. Hair roots are plucked, preferably from the scalp, using a pincet such as nr. 805 or nr. 970 Medicon Instruments (Tuttlingen, F.G.R.). Twenty-five follicles, from each individual, with visible bulb and sheath (Fig. 1A) are attached to

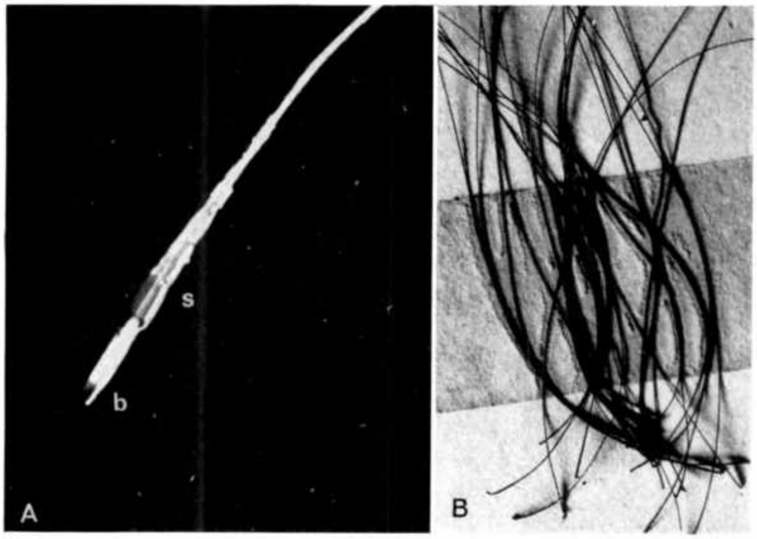

FIGURE 1. (A) Intact hair root from the human scalp. b = bulb; s = sheath (×40). (B) Hairs with bulb and sheath fixed on filter paper with tape for mailing.

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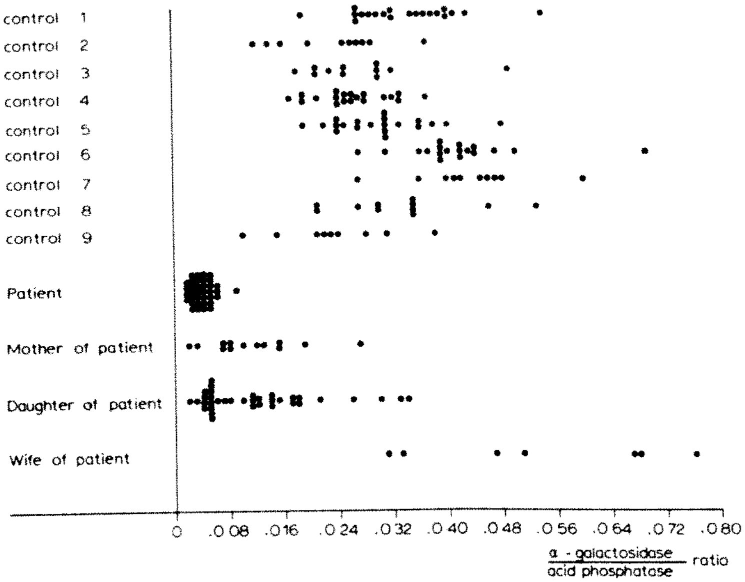

FIGURE 2. Enzyme activity ratios of hair root α-galactosidase and acid phosphatase. Each dot represents one single hair root.

filter paper with a strip of tape (Fig. 1B). In the present study they were put in an envelope and mailed from Vancouver (Canada) to Nijmegen (The Netherlands), where α-galactosidase and acid phosphatase activity measurements were carried out according to Vermorken *et al.* (1978).

A Fabry patient (diagnosis made by kidney biopsy and conventional α-galactosidase assay in white blood cells), heterozygotes and normal controls can clearly be discriminated on the basis of α-galactosidase/acid phosphatase activity ratios in their individual hair roots. In normal controls no ratios below 0·010 are observed. In the α-galactosidase deficient patient the ratios of the individual hair roots ranged from 0·002 to 0·009. In an obligate heterozygote (mother of the patient) activity ratios in the deficient as well as in the normal range were observed.

To demonstrate the presence of hair roots with normal and mutant enzyme activities we used between twenty and thirty hair roots. In practice this is sufficient and similar to the numbers used in studies by others (Gartler *et al.*, 1971; De Bruyn *et al.*, 1974; Grimm, Wienker & Ropers, 1976; Beaudet & Caskey, 1978; Ejiofor *et al.*, 1978). Although using another reference enzyme and avoiding protein determination in single hair roots, the present study is in agreement with previous studies (Grimm *et al.*, 1976; Beaudet & Caskey, 1978) that hair roots can successfully be employed in the diagnosis of heterozygotes for α-galactosidase deficiency.

Moreover, it shows that under the conditions described a 10-day interval between sampling and enzyme determination does not affect the assay. Similar observations have been made with hexosaminidase activity of mailed hair roots (Hösli *et al.*, 1977). Hair roots can be sent to Dr A.J.M. Vermorken, Department of Pharmacology, University of Nijmegen, Faculty of Medicine. Envelopes should be marked 'Fabry heterozygote detection'. Determinations are performed at cost price.

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Dithrocream in psoriasis

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A double blind clinical trial was carried out comparing 0·1% Dithrocream with 0·25% Dithrocream in patients with bilateral chronic plaque psoriasis.

Assessment of severity was based mainly on the extent of the plaques of psoriasis, categories being mild, moderate and severe. Patients with mild psoriasis were not admitted to hospital and did not, therefore, take part in this trial.

Of thirty consecutive in-patients one was excluded because of erythroderma. Twenty-nine patients were therefore entered for the trial, aged from 18 to 88 years. Five patients were withdrawn, three of these because of lack of response.

The trial was completed in twenty-four patients when they were discharged from hospital with their plaque psoriasis almost cleared; our criterion for 'almost clear' being when the lesions are flattened and impalpable but may still be visible. This clinical situation did not meet our normal criterion of complete clearance which has been defined as when the lesions are absent on both inspection and palpation (Farber & Harris, 1970). The nineteen patients with moderate psoriasis were able to be discharged in an average of 14 days (range 7–22 days); the five patients with severe psoriasis in an average of 23 days (range 12–35 days).

Previous treatment had little effect on the outcome. Eighteen patients had been applying a fluorinated steroid up to the time of admission.

Initial response with both strengths appeared good although this 'slowed down' in many patients and was particularly noticeable in three of the nineteen moderate cases, and four of the five severe cases. In all but one patient both sides responded in equal time although in two cases initial response was more rapid on the 0·25% side. Side-effects were not troublesome. Staining of the skin was common and noticeable in all but two patients. Ten patients stained equally on both sides, eleven stained more on the 0·25% side, and one stained less on that side. Staining of dressings and clothing appeared to be a minor problem only. Redness and burning occurred in a small minority and led to a temporary halt in treatment of four patients. All four responded well to re-starting treatment within 2 days. Scalps responded well initially although the response then appeared to slow so that complete clearing commonly coincided with flattening of the plaques. Dithrocream (0·25%) was supplied to patients on discharge from hospital for continued application to sites almost cleared and although out of the trial, it was found that in some instances this out-patient application did produce a complete clearance.

There have been suggestions that systemic absorption of dithranol may occur more readily from creams than ointments. For this reason four patients were selected at random for urinalysis and all showed a negative reaction for anthraquinones.

We conclude that these preparations are less potent in clearing psoriasis than are equivalent concentrations of dithranol in Lassar's Paste. We are satisfied that they are definitely effective, however, and produce slow but steady clearance of the disease in most cases. Their ease of administration and relative lack of staining of clothes and hair make them a preferred treatment for home usage in the

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maintenance of clearance of psoriasis achieved on in-patients by routine dithranol therapy. It is likely that when regularly applied by out-patients with moderately severe psoriasis, the same degree of slow clearance could be achieved by the majority.

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Cyclic AMP is decreased in mononuclear leukocytes from psoriasis patients

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Certain abnormalities of monocyte function have recently been described which seem to be characteristic of the psoriatic individual, occurring even in the absence of cutaneous lesions. These include increased chemotaxis (Krueger *et al.*, 1978), increased phagocytic capacity and increased bacteriocidal capacity (Bar-Eli *et al.*, 1979). Since all of these functions are under cyclic nucleotide control, it has been suggested that the observed changes may be the result of a reduced cyclic AMP/GMP ratio in the psoriatic monocyte (Bar-Eli *et al.*, 1979). Here we present direct experimental evidence to support this concept.

Blood (2·5 ml, EDTA anticoagulant) was obtained by venepuncture from psoriatic patients who were receiving topical therapy only, and from healthy controls who had no personal or family history of skin disease (Table 1). The washed cells were subject to isopycnic centrifugation on a pre-formed gradient of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden; starting density 1·077). The band of mononuclear leukocytes (MNL) was removed, washed twice and resuspended in 250 μl of medium containing 7·5 mmol/l theophylline. Cell density and percentage monocytes were measured electronically (Loos *et al.*, 1976). Stimulation with adrenaline, extraction of cyclic AMP and radioimmunoassay were as described for keratinocytes (Gommans *et al.*, 1979) with minor modification.

TABLE 1. Personal and clinical data regarding the subjects investigated, and the cyclic AMP levels (pmol/10^6 cells) in MNL preparations. TC = topical corticosteroid

Controls				Patients								
Age	Sex	Cyclic AMP levels		Age	Sex	Extent of lesions	Duration (years)	Treatment	Cyclic AMP levels	
		resting	stimulated						resting	stimulated
25	M	11·0	16·2	38	M	+	2	TC	9·5	15·6
30	F	7·3	18·5	28	M	+	1	None	5·7	14·7
28	M	9·2	16·5	65	F	++	10	TC	5·4	18·5
26	F	6·6	21·0	46	F	+	5	TC	4·5	20·0
32	F	8·9	29·9	30	M	++	2	TC	7·8	23·9
26	F	9·2	43·6	24	M	+	1	None	4·2	26·4
26	M	6·0	39·2	32	M	++	17	TC	7·7	33·9
33	M	7·4	31·9	47	M	++	25	TC	3·4	17·5
30	M	9·2	30·6	26	F	+++	1	Ingram	7·2	27·1
26	M	7·3	39·9	53	M	+++	21	Ingram	4·6	18·9
47	M	4·3	11·7	86	F	+++	34	Ingram	7·1	17·4
30	F	10·5	27·0	26	M	+	1	None	6·3	12·2
Mean		8·1	27·2						6·1	20·6
S.d.		1·9	10·4						1·8	6·2

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All manipulations were carried out in a basic medium of phosphate-buffered saline containing 2 g/l glucose and at a temperature of 0–4°C.

The average MNL content of the final supernatant was $2{\cdot}6 \times 10^6$ cells, representing an overall recovery of 42%, and contained a mean value of 30% monocytes. There was no significant difference in these figures between the control and psoriatic groups. The cyclic AMP measurements are presented in the table. The reduction in 'resting' levels in the psoriatic MNL is statistically significant ($P = 0.025$), and that following adrenaline stimulation is probably significant ($0{\cdot}1 > P > 0{\cdot}05$). The degree of stimulation by adrenaline (3·4 fold) was the same for psoriatic and for control cells. No correlation with extent or duration of the disease was found in the psoriatic group and no correlation with age or sex was seen in either control or psoriatic group. This last observation makes it unlikely that the lower cyclic AMP levels in the patients could have resulted from imperfect age- or sex-matching between the groups.

Our findings are clearly compatible with the postulate of Bar-Eli *et al.* (1979); unequivocal proof that the abnormality is located in the monocyte rather than the lymphocyte must await more refined cell separation techniques. The significance of a monocyte dysfunction in psoriatic patients is of course speculative, but an extremely interesting link has recently been provided by the discovery that the blood monocyte is a precursor of the epidermal Langerhans cell (Katz, Tamaki & Sachs, 1979). If the latter serves a regulatory function (reviewed by Wolff, 1972), it is clear that a genetic abnormality in this cell line could well have dramatic consequences for the skin.

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The Role of Langerhans cells in the Pirquet and Moro-Hamburger tuberculin reactions

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The epidermal Langerhans cells have lately been shown to have IgFc and C3 receptors (Stingl *et al.*, 1977) and to be the only cells in human epidermis expressing D/DR antigens (Klareskog *et al.*, 1977). Selective uptake of various contact allergens was demonstrated by Shelley & Juhlin (1977) and they suggested that the Langerhans cells form a reticulo-epithelial trap for external contact allergens. A frequent occurrence of apposition of mononuclear, probably lymphocytic, cells to Langerhans cells in contact allergic reactions (Silberberg, 1973) suggests a cooperation, and may indicate an antigen-presenting function of the Langerhans cells.

We have investigated the ability of Langerhans cells to present purified protein derivative (PPD) of tuberculin to purified autologous T lymphocytes. Using human epidermis from tuberculin positive individuals separated from dermis by means of a suction blister device and dissociated with trypsin, we obtained epidermal cell suspensions containing 3–5% Langerhans cells as judged by immunofluorescence staining with a rabbit anti-DR antiserum (Braathen & Thorsby, 1980).

Epidermal cells (Ec) or macrophages (Mø) were co-cultured with purified autologous T lymphocytes with or without PPD of tuberculin added in 5-day cultures. The macrophages and the purified T lymphocytes were made as previously described (Braathen & Thorsby, 1980). A total of fourteen different experiments were performed, and in Table 1 the results from three typical experiments are shown.

TABLE 1. Epidermal cell (Ec) and macrophage (MO) dependent T-lymphocyte response to PPD *in vitro*

	T* cells		T+Ec†		T+MØ‡	
PPD	—	+	—	+	—	+
Experiment 1	298±139§	254±91	220±79	4250±1062	186±26	3868±440
Experiment 2	199±83	169±47	240±176	7949±569	264±62	13239±1317
Experiment 3	206±78	295±96	106±32	6047±593	152±13	2611±613

* 5×10^4 T cells.
† 5×10^4 epidermal cells.
‡ 5×10^3 macrophages.
§ Mean c.p.m. ± s.e.

It can clearly be seen that epidermal cells can substitute for macrophages in the macrophage-dependent T-lymphocyte response to PPD. A strong T-cell response to PPD was obtained in cultures where epidermal cells or macrophages were present.

In cultures where epidermal cells pretreated with an anti-DR antiserum plus complement were used, no responses were obtained, while the responses obtained with epidermal cells pretreated with

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normal rabbit serum plus complement were equivalent to the responses obtained with untreated epidermal cells. The results of additional studies will be published elsewhere (Braathen & Thorsby, 1980).

These data indicate that HLA-DR positive Langerhans cells are able to present the bacterial antigen PPD of tuberculin in an immunogenic way to T-lymphocytes. This finding is of particular interest with regard to the Pirquet and Moro-Hamburger tuberculin reactions. In Norway the presently used modified von Pirquet test is performed by placing two drops of concentrated tuberculin on the volar aspect of the forearm. A scarification is then made in epidermis through each drop of tuberculin and the site left to dry for 5 min. The test reaction is read after 2–3 days, and the reading is based on the width of the erythematous and infiltrated area, measured in millimetres at right angles to the scar. A reaction of 4 mm or more is considered positive.

The Moro-Hamburger reaction is performed by applying tuberculin containing ointment to the skin. A positive tuberculin reaction is generally accepted to be a delayed type hypersensitivity reaction and the infiltrating mononuclear cells are predominantly T-lymphocytes (Braathen, Førre & Natvig, 1979).

Based on our findings we suggest that the tuberculin which is applied to the epidermis in the Pirquet and Moro-Hamburger tests is first bound to the Langerhans cells and then presented in association with HLA-D/DR molecules to the T-lymphocytes. We furthermore suggest that these events then constitute the afferent phase in the cutaneous cellular immune response to tuberculin in these tests.

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Effect of Gluten-free diet on recurrent aphthous ulceration

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Ferguson *et al.* (1976) found that eight out of thirty-three patients presenting with recurrent aphthous ulceration (R.A.U.) had jejunal mucosal features of coeliac disease; their oral ulceration remitted on a gluten-free diet. We have studied eighteen consecutive R.A.U. patients using standard haematological and biochemical parameters of malabsorption, including a xylose tolerance test. Twelve of the patients consented to a jejunal biopsy, following which they were placed on a gluten-free diet for 3 months. They maintained a daily record of the number of oral ulcers and a daily entry for the pain experienced on a visual analogue chart.

RESULTS

There was no haematological, biochemical or jejunal mucosal evidence of malabsorption or coeliac disease. Four patients discontinued the gluten-free diet. In the eight patients maintaining the gluten-free diet, there was a progressive reduction in the daily number of ulcers and pain experienced, but the differences were not statistically significant in this small group. Ferguson *et al.* (1976) would appear to have over-emphasized the importance of coeliac disease underlying R.A.U. and contrary to their suggestion there seems little justification for routinely subjecting R.A.U. patients to a jejunal biopsy. However, the apparent reduction in the incidence and painfulness of the R.A.U. merits further study.

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Comment

DO SYSTEMIC ANTIPRURITIC AGENTS WORK?

'Most of the agents recommended for the control of itching during the last two decades have not stood the test of time. Many have been discarded, and the continuing use of several of the remaining ones has been dictated not so much by the results obtained as by the desire to prescribe something for the complaining patient.'

Another 20 years have slipped past since Cormia & Dougherty wrote so bluntly about the subject in 1959. But has the picture really changed? The complaining patients still exist and skin clinics still echo to the rasp of fingernails on skin. There is obviously still a demand for at least one effective antipruritic agent, to be used in conditions like atopic eczema which cannot always be controlled by topical measures, and which cause long-term distress. This demand is matched by a mass swallowing of drugs reputed to have antipruritic effects—at least 50,000 prescriptions being issued each year in the United Kingdom alone. Yet despite this, skin text books are still coy about confirming that these drugs, usually antihistamines, do in fact suppress itching. Patients are sometimes less complimentary.

This review attempts the difficult task of assessing whether, 20 years on, the use of so-called antipruritic agents is still based upon inadequate evidence.

DEFINITIONS

Itching is easy to recognize but hard to define. The most widely accepted version, 'that unpleasant sensation which provokes the desire to scratch' (*Archives of Dermatology*, 1969), has at least the virtue of offering two ways of assessing itching. Itching itself is subjective, and can only be described in rather vague terms by the patient himself; and some may not be good at this. Itch, therefore, cannot be quantified in neat units. However the second component of the definition, scratching, can be measured; and there is now some evidence to support the commonsense notion that it correlates closely with itching (Felix & Shuster, 1975), but clearly this is not always the case. For example, when asleep, patients are not conscious of itching, though scratching continues unabated (Savin, Paterson & Oswald, 1973).

All trials of antipruritics, therefore, must be judged by the accuracy with which patients' opinions of their itching are recorded, or with which their scratching can be measured, or both.

For the purposes of this article an effective systemic antipruritic agent will be taken to be one which acts specifically to decrease itching in a way which is independent of other properties such as sedation, tranquillization, and the local blocking of chemicals released in the skin by itchy diseases. Most drugs recommended for itching do in fact have these other properties, which may be valuable clinically, but are not specific for itching.

It is this mixed bag of pharmacological actions which has made life so difficult for investigators in this field. Two simple examples will suffice to illustrate this. Tranquillizers act by making people worry less about things: under their influence patients may worry less about their itching, and report that it is less troublesome, even though they continue to scratch as much as before. Local actions must also be excluded; the itching of urticaria, for example, lessens when the eruption is controlled by an antihistamine, but this is not due to any specific antipruritic effect.

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Clinical trials must take these points into account, and as Winkelmann & Muller put it in 1964, 'The evaluation of the effectiveness of antipruritic therapy is an almost insurmountable task for clinical investigators.' It certainly demands ingenuity, rigorous methodology, and a variety of different approaches.

INVESTIGATIVE APPROACHES

Animal experiments

Even a parrot cannot describe its itching, and so only the measurement of scratching is possible. This has been tried, for example by Joglekar, Bhide & Balwani (1963), who used cowhage in an ointment base to make rats itchy, and then recorded scratching movements on smoked paper. The animals made this as difficult as they could by licking and biting at the itchy areas, and although the method was suggested as suitable for screening systemic antipruritic agents, it seems too far from clinical problems to be generally useful.

Mechanical itch production

Shelley & Arthur (1957) found that when a fine springy tungsten wire was applied to an itch point, it gave rise to a definite short itch after a period of about 2 s, probably without the liberation of itch producing chemicals. They applied this technique to the study of the physiology of itching, but it seems difficult to quantify.

Tronnier & Schule (1968) used a swinging tack made to move parallel to the skin surface by a magnetic oscillation system. They measured the threshold and duration of the itch, and the degree of erythema and wealing, using these to assess the effects of clemastine, diazepam, and a corticosteroid. The antihistamine, clemastine, perhaps not surprisingly, reduced both the itching and wealing; diazepam decreased the sensation of itching but not the wealing, and the corticosteroid had little effect on either. Although the authors felt this was a useful way of investigating systemic antipruritic agents, it has not been widely used, and perhaps the sensation produced was nearer to tickle than itch.

Electrical methods

Electrical stimuli have long been known to be capable of provoking itching, presumably by a direct action on skin nerve endings. Sherrington, in his classical work on the integrative action of the central nervous system, republished in 1947, laid the foundations of our knowledge of the physiology of the scratch reflex by studying the effects of electrical itch-provoking stimuli on animals. Later Bishop (1943) devised an electrical method which caused a sensation very like that of an insect bite, but Shelley & Arthur (1957) found this technique unsatisfactory because the intensity of the pruritus was faint. Their own technique with an inserted electrode and a square-wave stimulator provided a much more intense stimulus. Similar methods were used by Dalali & Adriani (1971) who showed that local anaesthetics could block this effect.

Edwards *et al.* (1976) found that with the method of Shelley & Arthur (1957) the cells and fine nerve endings surrounding the invasive electrode were rapidly cauterized so that itching could be produced only once or twice. They preferred saline-soaked gauze pads as electrodes, pointing out the advantages of their technique over the use of cowhage spicules, fine wire insertions, and the injection of chemicals. The stimulus could be easily quantified, and the itching ceased a few seconds after stimulation, so that repeated stimulations could be given. They used their method to study the effect of past skin disease, and of psychological stress, on itching, but not to study the actions of systemic antipruritic agents.

Chemical itch production

Keele & Armstrong (1964) have listed the ways in which pruritogenic substances can be applied to the skin. These include simple applications with inunction, iontophoresis, scarification, intra-epidermal and dermal injections, and applications to a blister base or to an area whose stratum corneum has been removed by stripping. The results vary according to the method of application. For example, Kirby, Heaton & Burton (1974) could not elicit itching with bile salts by prick or patch tests, and yet itching was easily provoked by applying them to the base of cantharidin-induced blisters. Histamine itself is another good example: it causes pain if injected intradermally at high concentration, and itching if applied to the epidermis.

These methods have been used to study the itch-producing qualities of many chemicals including histamine (Cormia, 1952), kallikrein and bradykinin (Hagermark, 1974), substance P (Hagermark, Hokfelt & Pernow, 1978), prostaglandins (Hagermark & Strandberg, 1977), bile salts (Varadi, 1974; Kirby *et al.*, 1974), cowhage spicules (Shelley & Arthur, 1957) and various proteolytic enzymes (Hagermark, Rajka & Bergquist, 1972; Hagermark, 1973; Rajka, 1968, 1969).

In some instances these findings can be related to the altered pharmacology of pruritic diseases, and here perhaps the best example has been the relationship between the pruritogenic qualities of different bile salts and the changes which actually occur in obstructive jaundice. But in most itchy conditions, the role of the substances known to be capable of inducing itching experimentally is still far from clear; and it is difficult to extend the argument to say that those drugs which help chemically induced itching will automatically help the itching of individual skin diseases since no doubt each will liberate a different set of pruritogens. For example, drugs which can be shown to reduce the itching of experimental histamine applications will not necessarily help the itching of, say, atopic eczema in which the role of histamine has not been established. In fact, Hagermark (1973) found that antihistamines inhibit histamine-induced itch but have no general antipruritic effect.

If these methods are to be used to test antipruritic agents, the way of assessing itching is important. The severity of pruritus is highly unreliable, and the two main alternatives are to measure the threshold concentration of the pruritogen at which itching is felt, or to record the duration of the itch. Here again confusion reigns: Cormia & Kuykendall (1954) felt that the end-point of itching, as contrasted to itch threshold, was not uniform, while Rajka (1968) felt that itch duration techniques were more appropriate and easier to perform than measurement of thresholds, and even more confusingly showed that there was no strict parallelism between itch duration and itch threshold even in the same individual. Cormia (1952) had earlier pointed out the importance other variables such as age (histamine induced weals being liable not to cause itching in subjects over 50), time of day (itch thresholds being decreased at night), psychological stress, and the site tested. All of these have to be taken into account in every study.

STUDIES OF ITCHING HUMAN SKIN DISEASES

The assessment of each systemic antipruritic agent seems to have passed historically through a number of stages: a first phase of clinical enthusiasm and reports of personal successes with small numbers of patients and a variety of skin diseases; a second phase of early clinical trials often not taking account of the many difficulties involved; a third phase of more elaborate trials, often with more disappointing results, and a growing awareness of placebo effects; and a fourth phase of the refinement of methods, expensive and carefully controlled trials, precise quantification, and attempts to separate the antipruritic effects from the other properties of the drugs.

Studies of trimeprazine illustrate all these phases very nicely. Early clinical reports were highly favourable; that of Hudson (1959) is representative, recording his gratifying experiences over a period of 5 months with many patients suffering from a variety of skin disorders. Quite clearly the drug deserved further evaluation.

Of several subsequent studies, that by Smith & Curwen (1961) was one of the best. They conducted a trial of trimeprazine and methdilazine against a placebo using the recently devised method of sequential analysis. Both drugs seemed to have a real effect on itching although the proportion of patients likely to be helped was, they felt, as low as 25%. They had, however, studied a mixed group of patients, some of whom had chronic urticaria, and perhaps not surprisingly, in view of the known antihistamine actions of both drugs, all of these preferred the active tablets. The inclusion of patients with conditions likely to be helped by this type of peripheral action makes the assessment of any central antipruritic action very difficult.

Other trials, for example that of Smith *et al.* (1962), were less precise statistically, using small numbers of patients some of whom had urticaria. Several trials gave no details of how itching was assessed, while others used methods as imprecise as nurses' impressions or the degree of excoriation of the skin. Hellier (1963) employed sequential analysis, excluded urticaria patients, and found no significant difference between the antipruritic activity of trimeprazine and amylobarbitone: however, Armitage (1963) himself, the inventor of sequential analysis, pointed out that his method had not been used correctly and the results were therefore invalidated.

Phase three was entered in 1964 when Epstein & Pinski, in a crisp short paper, demonstrated that no less than 66% of their itchy patients were helped by one or more of four differently coloured placebo tablets. To eliminate this effect, trials had to be more carefully controlled than ever before. Fischer (1968) confirmed the importance of the placebo effect and found no significant difference between the antipruritic activity of trimeprazine, cyproheptadine and a placebo although his patients did better with any of these three than with no medication at all.

We are now entering phase four. As the literature suggests that the antipruritic effect of trimeprazine may be small, studies to detect it have to be correspondingly precise, but this has proved to be difficult if patients' opinions are the only source of information. This applies particularly to trimeprazine, a phenothiazine derivative, possessing undoubted sedative and tranquillizing properties which will affect the degree to which patients are worried by their itching.

For this reason, recent investigators have concentrated upon measuring scratching rather than estimating itching itself. The obvious first step was to measure scratching during sleep since the patient can be conveniently tethered at night to the elaborate recording equipment, and since placebo effects do not operate during sleep (Adam *et al.*, 1976). However, the preliminary studies (Savin *et al.*, 1973, 1975) showed that the picture was more complicated than had been suspected as patients scratch more when awake than asleep, and that during sleep itself, scratching varied with the general reactivity of the individual sleep stages. This means that a drug such as trimeprazine, by virtue of its strong hypnotic properties, will tend to cut down scratching simply by making patients sleep more, and by making them spend more time in stages 3 and 4 of orthodox sleep when scratching is least frequent. Indeed this predictable hypnotic effect was found to be responsible for the modest reduction of nocturnal scratching by patients with atopic eczema given trimeprazine; but when the individual sleep stages were compared for scratch bout frequency, no significant reduction was found. Trimeprazine was in fact no more effective at suppressing nocturnal scratching than trimipramine, a drug with known hypnotic qualities, but one not thought to have any antipruritic effect (Savin *et al.*, 1979).

These complexities mean that studies of nocturnal scratching must employ EEG monitoring to separate hypnotic from specific antipruritic effects. Other studies, using ingenious gadgetry such as beds equipped with pressure sensitive transducers, and arm and leg meters to distinguish between scratching and general body movements (Felix & Shuster, 1975; Muston, Felix & Shuster, 1979), but without EEG control, have not been able to clarify this point.

DISCUSSION

Winkelmann & Muller (1964) were right: the assessment of any specific antipruritic action of drugs is an investigator's nightmare, and this is why the task has been shirked, leaving us with a literature which is smaller and less impressive than one has a right to expect of such a major problem.

Animal studies, while avoiding difficulties with placebos, cannot answer the problems of clinical dermatologists. Nor can data derived from experimental itch induction in man readily be translated into clinical law, though the electrical methods described above seem more promising than the chemical ones both in terms of accurate quantification and of avoidance of the problems caused by the local pharmacological actions of the drugs. However, at best these studies can only be a rapid way of screening drugs for clinical promise, and the only way of finding out if they really work is by trials on patients with itchy skin diseases.

All such clinical trials must be judged against the blue-print of the proper clinical investigation of antipruritic drugs devised by Cormia & Dougherty in 1959. The points they make are still valid but can now be updated in the light of further experience.

Series should include only cases suffering from the same skin condition and should exclude those likely to be improved by peripheral antihistamine effects such as urticaria. A chronic condition such as atopic eczema is ideal in that itching is unlikely to be self-limiting. Topical treatment should be unchanged for some time before and during the trial. A mixture of patients in hospital and at home is not suitable as the act of admission to the ward may itself influence the course of the disease. All studies must use placebo controls but even recent studies do not always avoid this trap (e.g. Baraf, 1976). The administration of the drug and the placebo must be strictly randomized.

The interpretation of treatment responses is still a major difficulty, not easily overcome even by the important-sounding 'daily pruritus score charts' and 'physicians global evaluations' of some recent studies. The measurement of scratching adds some precision but if done at night must be subject to EEG control. Results must be reproducible but this is not often possible in expensive and large trials. Chance variations must be eliminated as far as possible by careful design of the trial and by expert statistical analysis of the results.

It is depressing to find that few, if any, of the trials conducted over the last 20 years have measured up to Cormia & Dougherty's blue-print. The pervading impression of the literature is of corners cut, snags unseen, and a general desire to please. If a general rule emerges it is that the more precise the study, the less the specific antipruritic effect of a drug has been shown to be. The verdict at present must be the one peculiar to Scottish law—non-proven.

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British Journal of Dermatology (1980) **103**, 119.

Correspondence

Contractile elements in stratum granulosum

SIR, The demonstration by Shelley & Juhlin (*British Journal of Dermatology*, **102**, 123, 1980) that the stratum granulosum contains contractile elements is an important addition to our understanding of the late events in epidermal differentiation, and opens new avenues for investigation.

I would like to comment on their suggestion that the structural contractile element is keratohyalin, which appears to be based on the fact that keratohyalin granules are localized within the contractile layers. In fact, the stratum granulosum is also the sole location, within epidermis, of large masses and bundles of 4–6 nm microfilaments identical to the contractile elements in other non-muscle cells (Skerrow, 1978). These microfilaments have been observed in both human and bovine epidermis. They are present only in the peripheral cytoplasm and are not attached to desmosomes. The microfilament bundles are orientated in the direction of flattening of the granular cell: that is, parallel with the surface of the skin. The numbers of bundles seen in thin sections may be artificially low, as they are partially converted into disorganized masses by fixation with osmium tetroxide (Pollard, 1976). This lability, and low contrast, probably explains why the presence of these structures in the stratum granulosum is not well known.

In other tissues, disorganized or depolymerized actin bears no ultrastructural resemblance to keratohyalin. Furthermore, none of the extracted proteins believed to originate from keratohyalin (reviewed by Matoltsy, 1975) are similar to actin. I have previously suggested (Skerrow, 1978; Skerrow & Skerrow, 1980) that it is the microfilament bundles in the stratum granulosum which are involved in the active processes of cell spreading and surface unfolding which occur at this level. Contractile microfilament bundles, which are also recruited and orientated at a particular stage in differentiation, have analogous functions in embryonic tissue (Szollosi, 1970).

These ultrastructural observations thus complement the findings of Shelley & Juhlin, and would seem to provide a more likely candidate for the structural contractile elements than keratohyalin.

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Dilutions of corticosteroids

SIR, Dr Brian Watson and colleagues (1979) have given us a new insight into the principles of homeopathy. Whereas the established practitioners in this branch of medicine are content to use dilutions of about 10^{12} the authors have extended this principle to new extremes by observing the effects of steroids at concentrations as low as 10^{-49} M. Since Avogadro's number is 6×10^{23}, at 10^{-49} M one may expect to find one molecule of the active steroid in 1.7×10^{25} l. This is 1.7×10^{10} km^3. The volume of the oceans of the World is only 1.4×10^9 km^3.

These results imply that the pollution of the Earth's oceans by potent corticosteroids at concentrations previously thought unimportant may have profound effects on the physiology of marine life—an alarming but unlikely prospect.

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We quite agree with Dr Gaylarde. These findings must be artefactual and cannot be explained otherwise. We merely recorded what we actually found.

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Immunofluorescence findings in pityriasis lichenoides

SIR, referring to the immunofluorescence (IF) findings in pityriasis lichenoides (PL) by Clayton & Haffenden (1978) we would like to present our experiences in this field.

In three out of fifteen cases granular deposits of IgM and C3 were found along the epidermal basement membrane (BM) and/or in the walls of some of the papillary capillaries, while C3 alone could be found along the BM in seven cases in a granular or fibrillar pattern. In addition, fine fibrillar deposits of fibrin were seen in fourteen cases, focally localized along the BM, in the dermal papillae and around the small vessels of the subpapillary plexus. IgA, IgG, Clq and C4 were absent in all cases. So, in contrast to Thivolet, Faure & Chouvet (1979), our findings confirm the observation of Clayton & Haffenden that granular deposits of IgM and C3 can be found in PL. However, the incidence in our material (20%) is much lower than the 72% incidence found by these authors.

With respect to the diagnostic specificity of the direct IF picture for PL we have analysed the results of the direct IF examination on 800 skin biopsies from patients with various dermatological disorders. In 125 of these 800 cases, including various diagnoses such as erythema multiforme, prurigo, urticaria, parapsoriasis en plaques and polymorphous light eruption, an IF picture identical to that of PL was found. In most cases fibrin was found in the same pattern as described above. In addition, IgM/C3 granules were seen in the normal skin of patients with among others Hailey-Hailey disease, psoriasis and chronic discoid lupus erythematosus. These findings indicate that a direct IF pattern, characterized by focal localization of IgM/C3 granules in the BM region and the capillary walls, whether or not together with deposits of fibrin, is not specific for any diagnosis.

It is tempting to consider the granular deposits of IgM and C3 in PL as an indication for the involvement of an immune complex mediated mechanism, as circulating immune complexes have been detected in this disease (Clayton *et al.*, 1977). However, granular IgM/C3 deposits were found in the sun-exposed skin in more than 50% of healthy individuals by Baart de la Faille-Kuyper, van der Meer & Baart de la Faille (1974). Preliminary results of a similar investigation in our laboratory indicate that the same holds for the unexposed skin. Moreover, we did not find Clq or C4 in PL, which one would expect to be present with deposition of circulating immune complexes in the tissue. Therefore, in our opinion the results of the direct IF do not substantially sustain the hypothesis of an immune complex mediated pathogenesis in PL.

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British Journal of Dermatology (1980) **103**, 122.

Book Reviews

Dermatology, 3rd edn. J.S.PEGUM and HARVEY BAKER (1979) London: Baillière Tindall. Pp. 278, 106 figures, 12 colour plates. Price £4.95.

This little book of 278 pages has been mainly re-written in its 3rd edition. The last edition was in 1970. Some of the admired original phrases and descriptions by Dr R.M.B. McKenna and Dr E.L. Cohen have been retained. I enjoyed reading it and found it easy to read. It is aimed at medical students and doctors of all kinds (except dermatologists). There are 106 illustrations in black and white throughout the text and twelve colour plates have been placed in the centre of the book. It is impossible to illustrate a concise text completely and a small number of the black and white photographs are a little difficult to interpret. Most students today have an accompanying atlas of dermatology.

I would certainly add this paperback edition to the list of recommended texts not only for undergraduates but also for postgraduate students for the therapeutic commentaries are remarkably helpful.

ROBERT A.MAIN

Progress in Allergy, 27. Edited by K.ISHIZAKA, P.KALLOS, B.H.WAKSMAN and A.L.DE WECK (1980) Basel: S.Karger. Pp. 276.

This latest volume of *Progress in Allergy* has two reviews on clinical immunology and three on more fundamental topics.

Most useful for the dermatologist is 'Humoral and cellular aspects of amyloid disease: present status' by Scheinburg and colleagues. This is a concise account of the subject which sets out present knowledge of immunochemistry, amyloid-related serum components and cellular immunology. A final section gives a rational approach to therapy. The 'Immunology of nephritis' by Nielson and Phillips reviews comprehensively the various immunological mechanisms involved in the different varieties of nephritis. At the more basic level is a review of 'Local immunity and local immune responses' with much that is pertinent to dermatology—notably non-specific factors that protect mucosal surfaces, and humoral and cell-mediated immune responses on mucosal surfaces. 'Solubilization of antigen-antibody complexes' is a topic that may become increasingly important therapeutically, but the aspect that is dealt with here is the role of complement. Finally, there is an account of the interaction of cytotoxic T-lymphocytes and target cells.

J.J.CREAM

A Colour Atlas of Endocrinology. R.HALL, D.EVERED and R.GREENE (1979) Wolfe Medical Publications. Pp. 176. Price £18.

Endocrinological diagnosis is remarkably visual: indeed in this respect it is closer to dermatology than any other speciality. What a good idea, therefore, to produce a visual primer. Give the diagnostic images as pictures and scatter the key facts around them. The analogy is with the direct way of learning a language rather than the painful inefficient and, above all, unenjoyable method of grammar first.

Yet sadly, the book fails the admirable simplicity of this broad approach. And it fails for neglect of a basic law of communication about which the authors could not have known because I have only just made it up. It is the paradox of generalities which may be crudely stated as the broader the approach the more important the detail. All that means is that as the specific details become fewer their choice becomes more critical and inaccuracies become more apparent. The authors have failed on both counts. Many of the details—the key facts—are poorly selected and there are many, surprisingly many, errors amongst them.

Let me illustrate both these points by the skin—this is not unreasonable: the skin is a major target organ of hormones as well as being an endocrine organ in its own modest right. As an example of omission, on page 13, the fine wrinkled dry pale skin of hypopituitorism is presented but not explained, although the changes are quite well understood. On page 168 candidiasis is erroneously related to hypocalcaemia instead of idiopathic hypoparathyroidism, and itch and the parathyroids does not get a mention. A number of simple errors suggest either that they have been poorly copied from another source or that the subject was not really understood. For example, the skin is said to be thick in osteogenesis imperfecta; there is great confusion about acanthosis nigricans which in the diabetics of this book (page 148) masquerades under the name of 'pseudo- xanthoma nigricans'! The detail is particularly bad on cutaneous paraendocrinology, for example of chronic renal failure, intestinal malabsorbtion and anorexia nervosa (I recognized the photograph of the last of these as I had investigated the patient: she had scalp alopecia and lanugo hirsutes, hyperpigmentation and thin skin). In fact she is the only patient I have found with shear purpura from a dietarily induced reduction in skin collagen.

All this and more in a very cursory reading of the book! Yet with just a little more care the essential facts could have been selected and these small errors corrected out.

I find it difficult to explain the discrepancy between the idea of this book and its execution, particularly in the light of the experience and standing of its authors. But for that very reason I am sure that the next edition will make amends. I hope it does because the central idea is so very good I would like to be able to recommend it.

SAM SHUSTER

The Management of Sexually Transmitted Diseases. A Guide for the General Practitioner. EURO Reports and Studies, No. 12. R.R.WILLCOX (1979) Copenhagen: W.H.O. Pp. 79.

Dr Willcox has had a panel of European experts to review his manuscript. The book is printed in Denmark.

Reviewers often wonder who may benefit from a book, but no such doubt arises here. It is offered as 'a guide for the general practitioner' but should have a wider appeal, for example to medical students. It should also help the British dermatologist untrained in what used to be called venereology, or the casualty officer who may treat patients with sexually transmitted diseases before referring them to an expert.

This is in no sense a comprehensive textbook, but it is remarkable how much information is packed into its eighty pages. It contains up to date information on such subjects as treatment of gonorrhoea, aetiology of urethritis and serological tests for syphilis.

Where Dr Willcox discusses subjects common to both our disciplines, such as molluscum contagiosum, scabies or warts his emphasis may be different, but his advice is usually acceptable, although to inject 1:1000 adrenaline under multiple perianal warts might prove lethal rather than useful.

This book is really too good for minor criticism. There are, however, numerous and irritating mistakes which could surely have been avoided by careful proof reading. These include amphoteracin, sensitity and indomethacin, indamethacin and indometacin, all on the same page.

DENIS SHARVILL

Dermabrasion and Chemical Peeling. J.W.BURKS (1979) Springfield, Illinois: Charles C.Thomas. Pp. 236. Price $22.50.

This book is based on the author's experience of over 25,000 dermabrasions. He traces the history of the operation and describes various techniques and indications for dermabrasion. However, his enthusiasm for the procedure, extending to its use in skin tumours and leg ulcers, may seem excessive and not all people in this country would agree for example that adenoma sebaceum responds most favourably to dermabrasion.

The book will be of value to anyone practising dermabrasion, but for the average British dermatologist cannot be recommended. However, plastic surgeons may find the book useful and the chapter on chemical peeling contains helpful hints for dermatologists wishing to practice this procedure.

W.J.CUNLIFFE

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News and Notices

Occupational Cutaneous Hazards and Diseases

The National Institute for Occupational Safety and Health (NIOSH) is currently inviting applications for research and demonstration grants to study a variety of issues and problems surrounding occupational cutaneous hazards and diseases.

Further information may be obtained from: Roger A. Nelson, Research Grants Program Officer, National Institute for Occupational Safety and Health, 5600 Fishers Lane, Room 8–63, Rockville, Maryland 20857, U.S.A.

History of Dermatology Prize

The History of Dermatology Club of the American Academy of Dermatology announces the Samuel J. Zakon Award in the History of Dermatology. This prize will be awarded to the dermatologist in training or in practice who submits the best essay on a subject related to the history of dermatology.

All papers should be submitted to Lawrence Charles Parish, M.D., President, History of Dermatology Club, 1601 Walnut Street, Philadelphia, PA 19102, U.S.A. by 15 October 1980.

The winner will receive his or her award at the History of Dermatology Club's Annual Meeting in December 1980.

This award was created by Mrs Samuel J. Zakon, in memory of her husband, who was a noted historian of dermatology and the first Historian of the American Academy of Dermatology.

Mr and Mrs J.N. Taub International Memorial Award for Psoriasis Research

The Department of Dermatology, Baylor College of Medicine, announces that the 1979 winners of the Mr and Mrs J.N. Taub International Memorial Award for Psoriasis Research are: Thomas B. Fitzpatrick, M.D., John A. Parrish, M.D., and Klaus Wolff, M.D. for their work relating to PUVA. These researchers' contributions relate not only to the treatment of psoriasis but further our knowledge of photobiology and photochemotherapy.

The recipients are chosen by an anonymous seven-member committee.

University Hospital of Wales—Dermatopathology Course

A dermatopathology course will be held at the University Hospital of Wales in Cardiff, September 23–26 1980, run jointly by the Dermatology and Pathology Departments.

It is intended for dermatology and pathology students in training. The course fee is £40, and further information and application forms may be obtained from: Dr A.G. Knight, Consultant Dermatologist, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.

International Congress

The 2nd International Congress for Dermatologic Surgery will be held in Paris, 28th September to 1st October 1980.

Further information may be obtained from: Secretariat, 2e Congrès International de Chirugie Dermatologique, 154 rue Armand Silvestre, 92400 Courbevoie, France.

Practical Dermatology Course for Registrars and Senior Registrars, Oxford, 9–10 October 1980

The course, which is open only to trainees, will entail instruction on rational investigation and practical management in the fields of vascular diseases, psoriasis and related diseases, cryosurgery and disorders of hair and nails. Each section will comprise lectures and clinical demonstrations.

For further details please apply to Dr Ryan and Dr Dawber, 'October Course', Slade Hospital, Headington, Oxford. Tel: Oxford 64841, Ext. 499.

Practical Skin Pathology Course

A 6-day course in Practical Skin Pathology will be held at the Antlers Plaza Hotel in Colorado Springs, Colorado, 12–17 October 1980. The course is designed as a comprehensive, practical review and update of dermatopathology for pathologists and dermatologists. There will be thirty-six formal lectures, sixteen question and answer periods, one panel discussion, four supervised microscopic workshop sessions for the study of over 400 new microscopic slides, and a generous amount of time for independent study or relaxation. New features this year include an approach to the histopathological diagnosis, special stains and artefacts, electron microscopy, immunofluorescence microscopy, a session on differential diagnosis, and a stump-the-faculty panel. For further information, please write to Loren E. Golitz, M.D., Skin Pathology Course, 11466 East Arkansas Avenue, Aurora, Colorado 80012, U.S.A. or phone (303) 893-7108.

Cleveland Clinic Foundation Dermatopathology Workshop

A Dermatopathology Workshop will be held at The Cleveland Clinic Foundation on 11th October 1980. The course directors are Dr W.F. Bergfeld and Dr B. Michel. Further information may be obtained from: Dr W.F. Bergfeld, M.D., Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44106, U.S.A.

Anthralin (dithranol) Symposium

A symposium on current concepts in the mode of action of Anthralin in the treatment of psoriasis will be held in Sophia Antipolis, France, 17–18th October 1980.

This is sponsored by the E.S.D.R. and Faculté de Medécine de Nice, and organized by Professors M. Greaves, L. Juhlin and H. Schaefer. Further information may be obtained from: CIRD—Centre International de Recherches Dermatologiques, Sophia Antipolis, 06560 Valbonne, France.

University of Cambridge

The annual Introductory course on the biology of the skin will be held in Fitzwilliam College, Cambridge, on 9–12 December 1980.

Details may be obtained from The Postgraduate Medical Centre, Level 6, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

The B.A.D. Investigative Group—Travelling Fellowship

A Fellowship of £200 has been established and will be awarded annually to meet the expenses of an Investigative Group member's attendance at the annual meeting of the European Society for Dermatological Research in Amsterdam.

The Fellowship will be awarded on the basis of a paper submitted for oral presentation at the winter B.A.D. Investigative Group meeting. The applicant for the Fellowship should submit his or her summary to the B.A.D. Investigative Group in the usual way, and include a one page curriculum vitae and a statement that he/she wishes to be considered for the Fellowship. In the case of multiple-author papers only the author delivering the paper in person will be considered.

The first Fellowship will be awarded on the basis of summaries submitted by October 25th, 1980, for selection for the 1981 Investigative Group meeting, to be held in Cardiff on January 23rd and 24th, 1981. Nine copies of a 250 word abstract containing a statement of the purpose of the study, methods used, and a summary of the results presented in sufficient detail to support the conclusions should be submitted to Professor R.M. Mackie, University Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU, from whom further details can be obtained. Supplementary information, such as electron micrographs and tables, can be submitted at the discretion of the author for the use of the selection committee. Please underline the name of the author who will present the paper.

Hawaiian Dermatology Seminar, Maui, Hawaii

This course is oriented primarily to dermatologists involved in patient care and will be held in Maui, 6–11 February 1981.

This postgraduate course is sponsored by the Northwestern University, Cleveland Clinic, and University of California (San Francisco) Departments of Dermatology. Speakers include the faculty of these departments and numerous guest faculty.

A post-convention seminar will follow in Tahiti.

For registration information and a preliminary programme, write to: Department of Dermatology, Northwestern University Medical School, 303 E. Chicago Avenue, Evanston, IL 60201, U.S.A.

Dystrophic Epidermolysis Bullosa Research Association

This Association was founded 2 years ago, to raise funds for research into the cause, nature, treatment and cure of all types of epidermolysis bullosa. Applications for grants for research into this disease can now be considered.

Further information may be obtained from the secretary: Mrs W. Foster, 38 Cornwall Avenue, Clayton, Newcastle-under-Lyme, Staffs.

APPOINTMENTS

Devon Area Health Authority

Dr Hilary Hubbard has been appointed Consultant Dermatologist to North Devon, based on Barnstaple, and to Exeter. She was inspired by the undergraduate dermatology teaching at the Royal Free Hospital, London, qualifying M.B., B.S. and M.R.C.S., L.R.C.P. in 1970. She became a member of the Royal College of Physicians and Registrar to the Manchester Skin Hospital in 1975. Her training continued as Senior Registrar in Manchester, where the main emphasis has been on therapeutics.

Her current interests are the effects of light on the skin, and the absorption of topical corticosteroid preparations.

University Hospital, Nottingham

Dr Leslie Millard has been appointed Consultant Dermatologist based at the University Hospital, Queens Medical Centre, Nottingham. Dr Millard qualified M.B., Ch.B. (Sheffield) in 1968 and M.R.C.P. (U.K.) in 1971. After a registrar post in the Rupert Hallam Department of Dermatology in Sheffield he was appointed Tutor in Dermatology to Leeds University based at the General Infirmary at Leeds in 1973. In 1978 he became Consultant Dermatologist to the Rotherham Area Health Authority.

For his research into cutaneous lupus erythematosus he was awarded the degree of M.D. in 1980. His other research interests include the use of methotrexate in the treatment of psoriasis and the treatment of leg ulcers. He has produced tape-slide instructional programmes and is the co-author of a book on the treatment of skin disease.

The Royal College of Physicians, London

The following dermatologists have recently been elected to The Fellowship of the Royal College of Physicians, London: Dr Julia P. Ellis. Dr Ellen S. Emslie, Dr A.E.D. Navaratnam and Dr I.A. Stahle.

Another Munksgaard Journal

Contact Dermatitis

Environmental and Occupational Dermatitis

Editor
C. D. CALNAN, M.D., London

Contact Dermatitis is designed primarily as a journal for clinicians who are interested in various aspects of environmental dermatitis. This includes both allergic and irritant (toxic) types of contact dermatitis, occupational (industrial) dermatitis and consumers' dermatitis from such products as cosmetics and toiletries.

The journal aims at promoting and maintaining communication among dermatologists, industrial physicians, allergists and clinical immunologists, as well as chemists and research workers involved in industry and the production of consumer goods.

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Journal of Cutaneous Pathology publishes manuscripts of the highest scientific quality in the field of cutaneous pathology and aims at promoting and maintaining communication between clinicians and research workers. Cutaneous pathology is interpreted to mean diseases of the epidermis, dermis and cutaneous appendages. Original scientific articles on diagnostic and experimental cutaneous pathology are invited. Clinico-pathological studies will also be considered for publication. Manuscripts based on light and electron microscopy, histochemistry, microbiology, biochemistry, immunology, pharmacology, tissue culture, embryology and genetics are welcome, provided they advance the science of cutaneous pathology.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents.*

Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations,* A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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British Journal of Dermatology

The British Journal of Dermatology is owned by and is the official organ of the British Association of Dermatologists. It is also the official organ of the Nederlandse Vereniging voor Dermatologie en Venereologie (Netherlands Association for Dermatology and Venereology).

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2. Becker, F.T., Cutis, 1974, **14**, (4), 610
3. Cullen, S.I. and Cohan, R.H., Cutis, 1976, **17**, 1208

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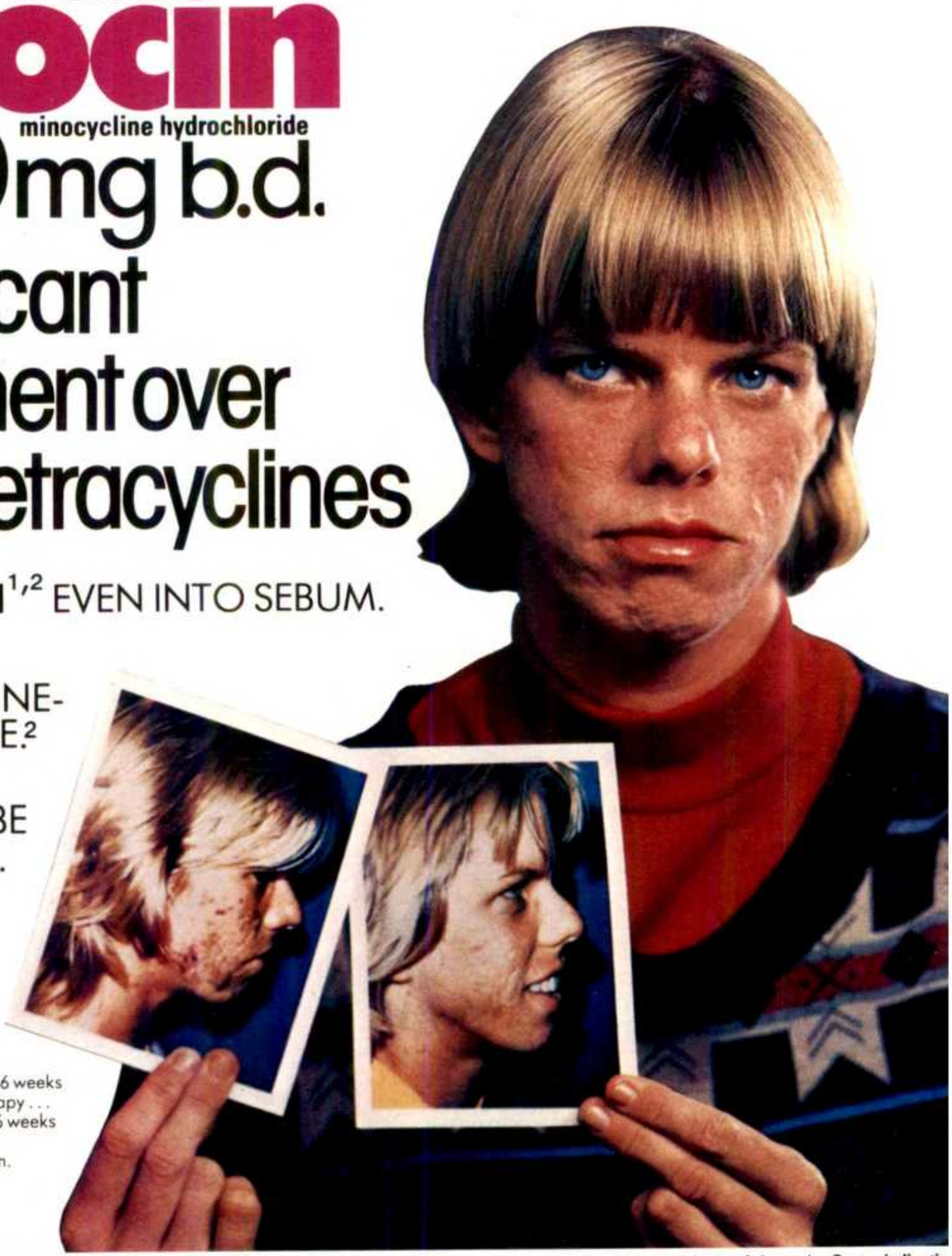

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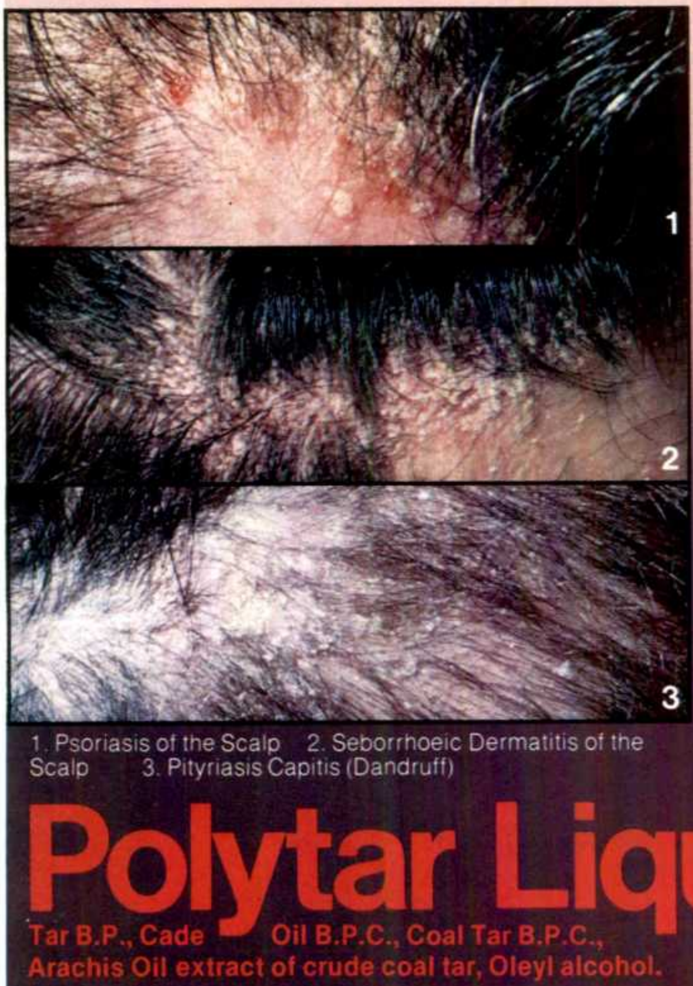

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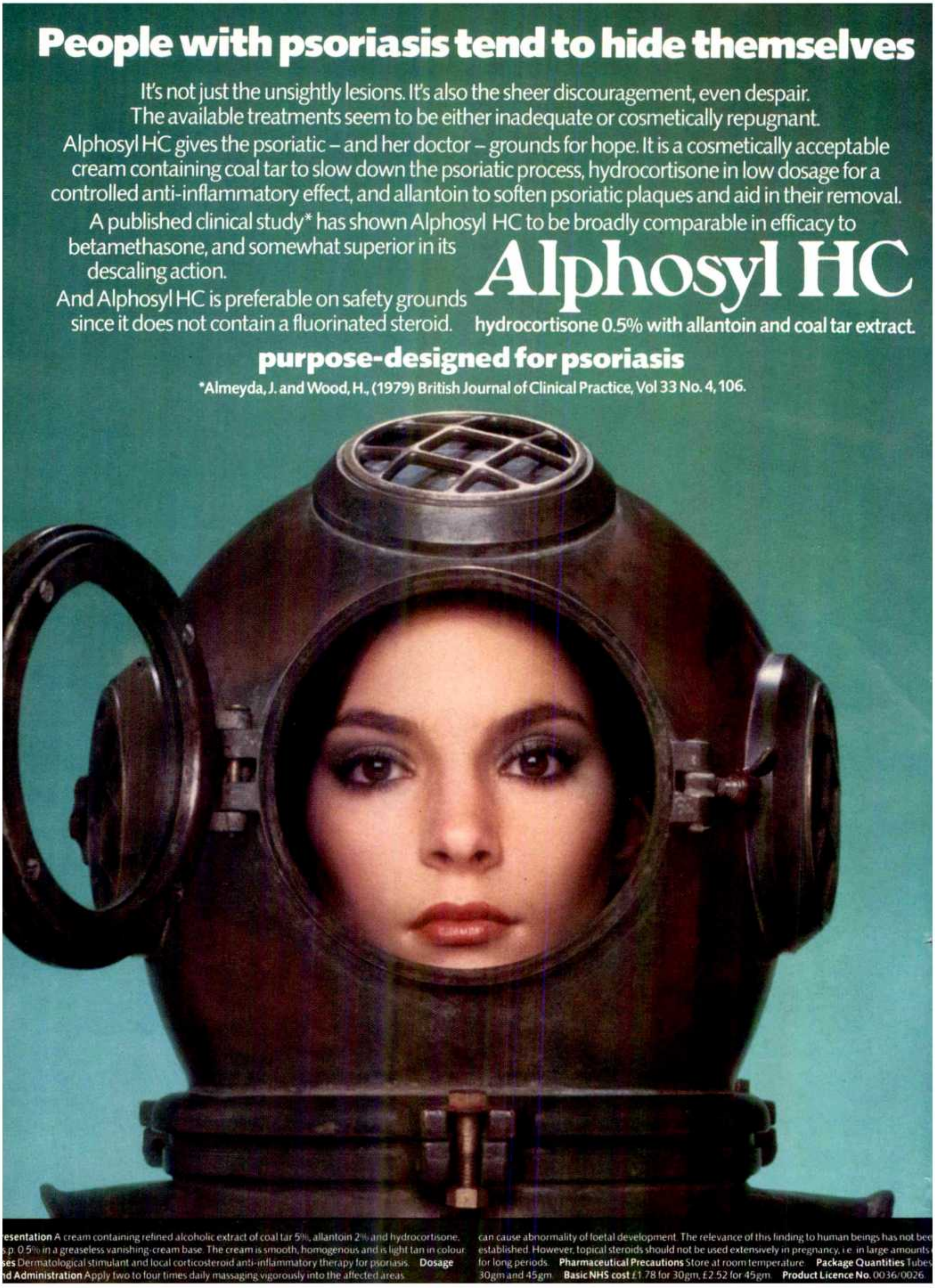

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*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol 33 No. 4, 106.

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Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

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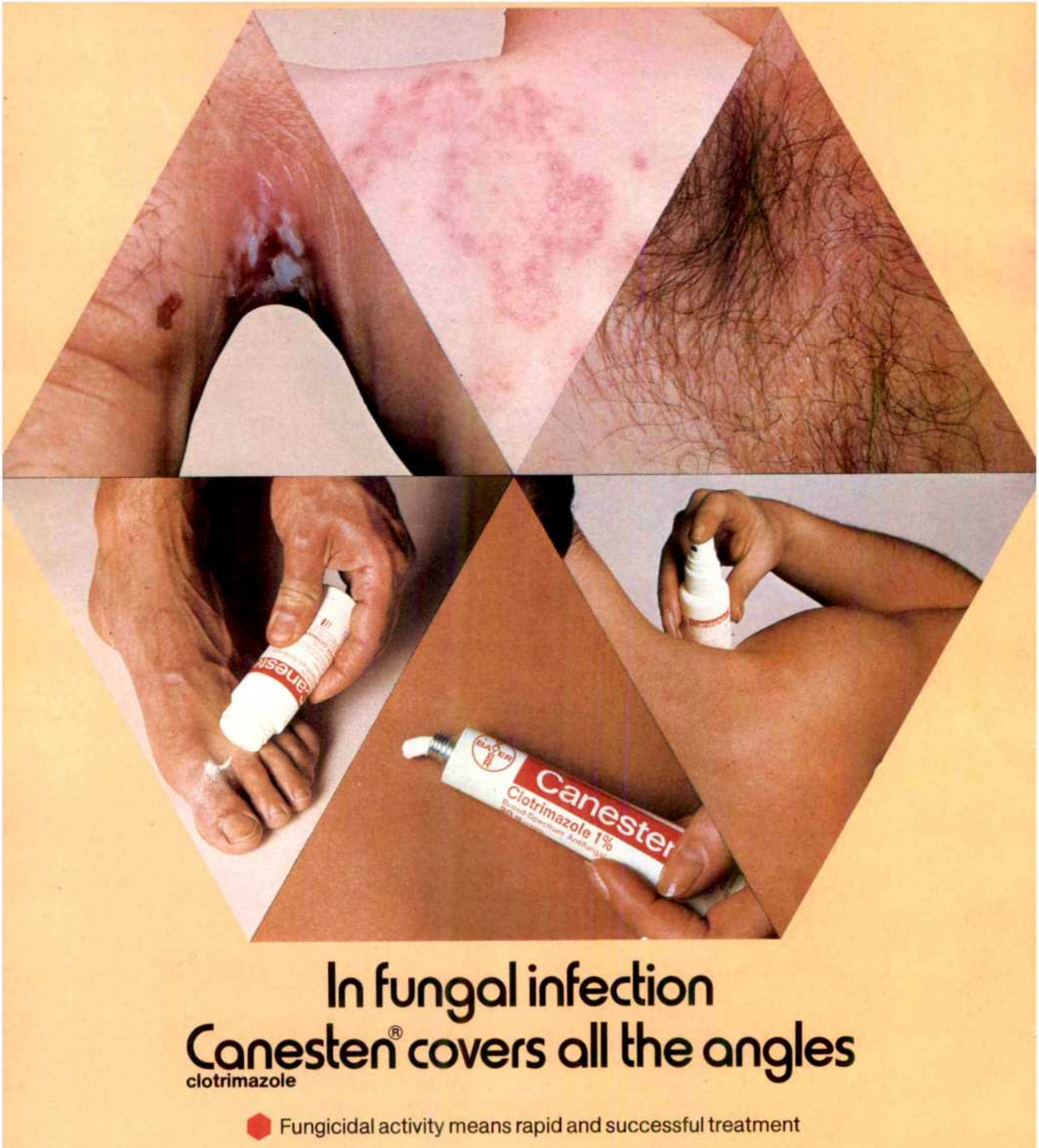
(exclusive of VAT)	£9·80	
Bottle of 100 tablets (500mg)		125mg: 4/5060 500mg: 4/5061

Glaxo

Full prescribing information on Grisovin (trade mark) is available from:

Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE



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clotrimazole

- Fungicidal activity means rapid and successful treatment
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escribing information: Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white powc
ntaining 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections. **Dosage and Administration** Crea
omiser Spray Apply to the affected area 2 - 3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylac
ainst re-infection. **Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near a
ked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled. **Product Licence Numbers** Cream: PL 0010/0016
omiser Spray: PL 0010/0060 Powder: PL 0010/0067 **Basic NHS Prices** Cream: 20g, £1.52 50g, £3.55 Atomiser Spray: 40ml, £4.08 Powder: 30g, £1.10. Further information
available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. Distributed in the Republic of Ireland by
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(clobetasol propionate)

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Prescribing information

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Side effects
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism.
This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing

Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.
In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom

adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.
Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.
Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications
Rosacea, acne and peri-oral dermatitis. Bacterial, fungal or viral diseases of the skin.

Basic NHS cost (exclusive of VAT)
Dermovate Cream or Ointment 25 gram tubes £2·07 (also available in 100 gram tubes).

Presentation	**Product Licence number**
Dermovate Cream	4/0219
Ointment	4/0220

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Chlorquinaldol is effective against staphylococci streptococci and other gram positive organisms which are usually found in bacterially infected skin conditions(1)

✱Antifungal

Chlorquinaldol has been shown to be more effective than clioquinol in an in vitro study(2) of activity against Candida albicans. Chlorquinaldol is also effective against trichophyton and microsporum species.

✱Low Sensitization(3)

✱Highly acceptable(4)

Locoid C

Hydrocortisone 17 butyrate 0.1% chlorquinaldol 3.0%

When infection is present or suspected.

References:

(1) Amer Pract. & Digest of Treat (1955) 6 8
(2) Transact New York Acad of Sci (1955) 10 161
(3) Arch. Derm (1972) 106 335
(4) Rocky Mtn. Med J (1955) 52 6

Prescribing Notes

Dosage & Use. Apply to the affected part two to four times a day until the infection is resolved. If the underlying condition persists treatment may be continued for up to 14 days and no longer. No more than 60g may be applied during this period. Do not use under occlusion in the presence of infection.

Contra Indications. As with all topical steroids, Locoid C preparations are contraindicated in the presence of viral, tubercular or syphilitic lesions.

Other Precautions. Avoid contact with the eyes. Not to be used in pregnancy in large amounts or for long periods.

In infants, avoid long term continuous therapy, especially avoid occlusion.

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P/L nos Locoid C cream 0166/0056
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Prescribing information

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conditions.

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Contra-indications

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skin.

Precautions

In infants and children, long-term
continuous topical corticosteroid th
should be avoided where possible, a
adrenal suppression can occur. In in
the napkin may act as an occlusive
dressing, and increase absorption.
Appropriate antimicrobial therapy sh
be used concurrently whenever treat
inflammatory lesions which have bec
infected.
Any spread of infection requires
withdrawal of topical corticosteroid
therapy and, possibly, commenceme
systemic chemotherapy.
As with all corticosteroids, prolonge
application to the face is undesirable
Topical steroids should not be used
extensively in pregnancy, i.e., in larg
amounts or for prolonged periods.

Side effects

In the unlikely event of signs of
hypersensitivity appearing, applicatio
should stop immediately. If large area
were to be treated, some patients co
absorb sufficient steroid to cause tra
adrenal depression despite the low d
of systemic activity associated with
clobetasone butyrate.
Local atrophic changes could occur
moisture increases absorption of ste
but only after prolonged use.

Basic NHS cost (exclusive of VAT)
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Product Licence numbers
Cream: 4/0233
Ointment: 4/0254

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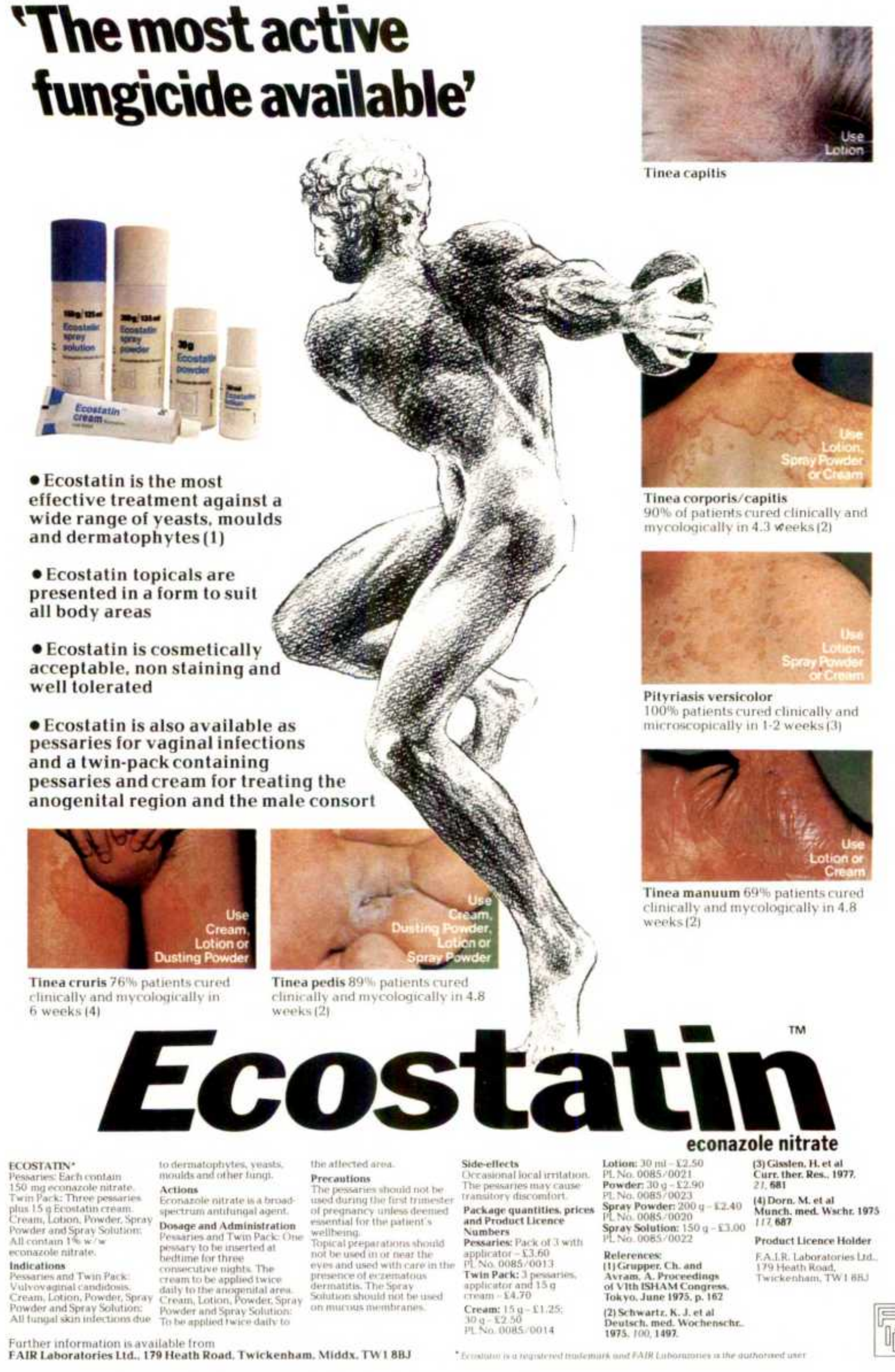
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Powder: 30 g – £2.90
PL No. 0085/0023
Spray Powder: 200 g – £2.40
PL No. 0085/0020
Spray Solution: 150 g – £3.00
PL No. 0085/0022

References:
(1) Grupper, Ch. and Avram, A. Proceedings of VIth ISHAM Congress, Tokyo, June 1975, p. 162
(2) Schwartz, K. J. et al Deutsch. med. Wochenschr., 1975, *100*, **1497.**
(3) Gissien, H. et al Curr. ther. Res., 1977, *21*, **681**
(4) Dorn, M. et al Munch. med. Wschr. 1975 *117*, **687**

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PRESCRIBING INFORMATION

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Benzoyl peroxide 10%, Potassium hydroxyquinoline sulphate B.P.C. 0.5%.

Indications:
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Dosage:
By gentle massage over all the affected area, two or three times daily.

Contraindications, Warnings etc:
Patients with known sensitivity to either of the active constituents. In a few isolated cases overreaction to the preparation may occur. To minimise this possibllity, select a small area of skin behind the ear, apply the preparation and leave for twelve hours. If severe irritation or pronounced redness occurs do not proceed.
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P.L. No: **Quinoderm Lotio-Gel 0291/0007**
Quinoderm Cream 0291/5000

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Contraindications, Warnings etc:
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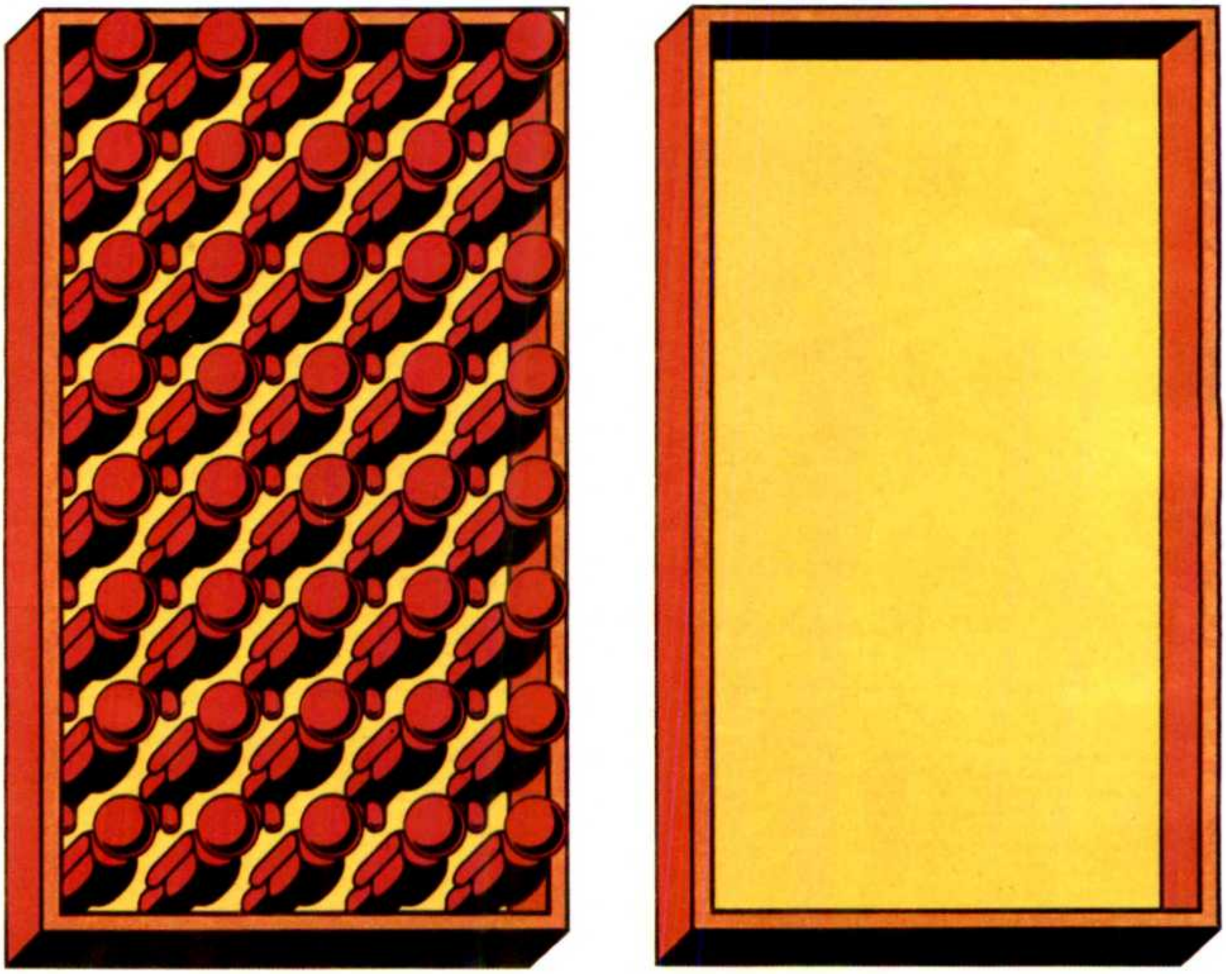

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Presentation
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Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

Indications
For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use
Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications
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Precautions
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Basic N.H.S. prices:
Dithrocream 0.1% 50gm tubes £1.81
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Dithrocream Forte 50gm tubes £2.19

Product Licence Nos.

Dithrocream 0.1%	PL 0173/0029
Dithrocream 0.25%	PL 0173/0028
Dithrocream Forte (0.5%)	PL 0173/0027

References:
1. *British Journal of Dermatology*, 1979, **100**, 475

Full prescribing information is available from
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Dithrocream is a registered trade mark.

Now available in three strengths

British Journal of Dermatology (1980) **103**, 127.

Clinical and Laboratory Investigations

Epidermal lipid biosynthesis in acne

SAM SHUSTER, MARY F.COOPER, D.McGIBBON
AND P.D.WILSON

University Department of Dermatology, Newcastle upon Tyne NE1 4LP

Accepted for publication 16 November 1979

SUMMARY

Lipid biosynthesis from glucose was increased in severe acne both in dermis and in epidermis which included the top portions of the pilosebaceous follicles. Both tissues retained their characteristic lipid labelling pattern. The magnitude of the increase in dermis and epidermis was similar, suggesting that epidermis may be subject to the same sebotrophic-lipogenic stimulus which affects sebaceous glands.

It is now well established that the severity of acne is related to sebaceous gland activity (Cunliffe & Shuster, 1969; Burton & Shuster, 1971; Cotterill, Cunliffe & Williamson, 1971; Pochi & Strauss, 1974). Sebaceous glands are an active site of lipogenesis, and dermal lipogenic activity is increased in severe acne (Cooper, McGrath & Shuster, 1976a; Cooper *et al.*, 1976b; Cooper, McGibbon & Shuster, 1977).

Blockage of the sebaceous duct has also been considered as an aetiological factor in acne, and it is widely believed that this is due to a factor produced by the action of bacteria on the sebum, which induces keratinization of the duct. Since lipids are normally synthesised by epidermis and pilosebaceous ducts, and many disorders of keratinization are accompanied by changes in lipogenesis (Cooper, McGrath & Shuster, 1976c; Ziboh & Hsia, 1972; Cooper, McGibbon & Shuster, 1978a; Cooper *et al.*, 1978b; Cooper, Wilson & Shuster, 1978c; Dykes & Marks, 1978), we examined the alternative possibility that both duct and gland are subject to a common lipogenic stimulus.

MATERIALS AND METHODS

The patients in this study were forty-seven males aged from 17 to 31 years, divided into two groups as follows: (a) mild acne (clinical grades 0–1) ($n = 11$); and (b) severe acne (grades 2–4) ($n = 36$) (Burton *et al.*, 1971). Subjects with disorders which might affect epidermal lipogenesis (e.g. psoriasis, atopic eczema) were excluded. Dermal and epidermal lipogenesis was measured as already described (Cooper, McGrath & Shuster, 1976a, c): 4 mm high-speed punch biopsies of lesion-free skin were taken from the mid point of the inner margin of the scapula, under lignocaine anaesthesia, and

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incubated for 3 h at 37°C in Krebs Ringer bicarbonate containing antibiotics (penicillin and streptomycin, each 100 μg/ml) and 2 mM [U-^{14}C] glucose (5 mCi/mmol), under $O_2+5\%$ CO_2. After incubation the tissue was treated with 0·5% acetic acid at room temperature for 2·5–3 h to loosen the epidermis, which could then be peeled away. Histological examination of epidermis and dermis treated in this way showed the epidermis to be completely separated at the dermo–epidermal junction together with the top portions of some of the pilosebaceous follicles, leaving the follicles and sebaceous glands intact in the dermis. Total lipids were extracted from dermis and epidermis and analysed by thin-layer chromatography and scintillation counting (Cooper *et al.*, 1976c).

Statistical methods used were the F-test for comparison of variances and Student's '*t*'-test, modified where necessary for $s_1^2 \neq s_2^2$.

RESULTS

Incorporation of ^{14}C into total lipids was increased in severe acne both in dermis and in epidermis, which included the top portions of the pilosebaceous follicles (Fig. 1). In epidermis this increase was highly significant ($P<0\cdot005$), and there was also a significant increase in the variance ($P<0\cdot05$). In dermis, lipid labelling was increased to a similar extent (about 60%), but because of larger standard errors this difference was only just significant ($P<0\cdot05$).

The pattern of ^{14}C incorporation into different lipid classes was distinctive in dermis and epidermis (Table 1), with epidermis showing more incorporation into polar lipids and sterols, and dermis showing more incorporation into triglycerides, squalene, and the combined wax monoester+sterol ester fraction. There were no significant differences in dermal labelling pattern between mild and severe acne (Table 1). Epidermal labelling also showed no differences, except for a small increase in free fatty acid labelling in severe acne ($P<0\cdot02$), and a relative decrease in the radioactivity chromatographing between triglyceride and wax monoesters ($P<0\cdot025$) (Table 1).

Figure 2 shows the relationship of dermal to epidermal lipogenic activity. There was a significant positive correlation ($P<0\cdot001$), but with considerable scatter ($r = +0\cdot496$).

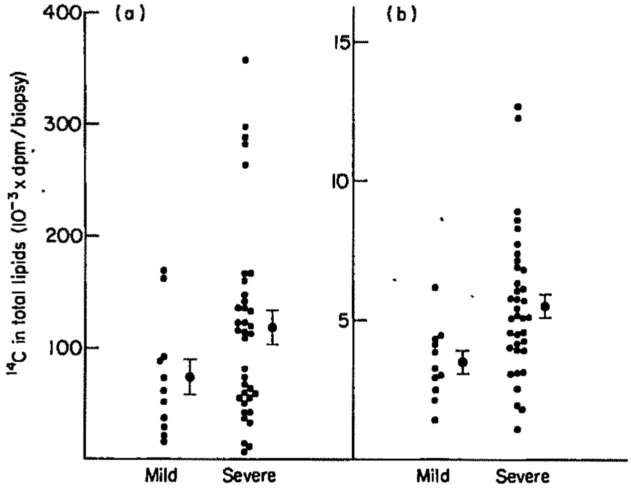

FIGURE 1. ^{14}C incorporation into total lipids of (a) dermis and (b) epidermis of skin biopsies from subjects with mild acne (grades 0–1) and severe acne (grades 2–4). Results are shown as individual values and as means ± s.e. mean.

TABLE 1. Incorporation of ^{14}C into lipid classes of dermis and epidermis, expressed as a percentage of total lipid labelling for each tissue (mean ± s.e. mean)

Lipid class	Dermis Mild acne (n = 11)	Dermis Severe acne (n = 36)	Epidermis Mild acne (n = 11)	Epidermis Severe acne (n = 36)
Polar lipids	7·74 ± 0·71	8·16 ± 1·06	54·08 ± 2·13	49·79 ± 2·34
Monoglycerides	1·53 ± 0·46	1·59 ± 0·22	4·63 ± 0·81	5·72 ± 0·48
Free Fatty Acids	2·91 ± 0·30	3·68 ± 0·37	5·16 ± 0·58	7·43 ± 0·65*
Sterols	0·82 ± 0·09	1·12 ± 0·16	4·99 ± 0·46	5·79 ± 0·52
1,2-diglycerides	3·56 ± 0·78	3·82 ± 0·38	7·61 ± 0·97	8·08 ± 0·58
1,3-diglycerides + fatty alcohols	2·09 ± 0·54	2·55 ± 0·24	2·92 ± 0·29	3·73 ± 0·39
Triglycerides + glyceryl ether lipids	50·91 ± 3·32	50·52 ± 1·64	11·61 ± 0·97	13·59 ± 1·52
'Wax diesters'	0·79 ± 0·11	1·29 ± 0·44	2·22 ± 0·55	0·72 ± 0·15†
Wax monoesters + sterol esters	9·26 ± 0·84	8·84 ± 0·69	1·59 ± 0·34	1·28 ± 0·29
Squalene	19·85 ± 1·81	18·37 ± 1·23	2·46 ± 0·48	2·56 ± 0·46

'Wax diesters' = material chromatographing between triglycerides and wax monoesters, i.e. cochromatographing with the diester waxes found in animal surface lipid. * Severe acne different from mild acne at $P < 0·025$; † severe acne different from mild acne at $P < 0·02$.

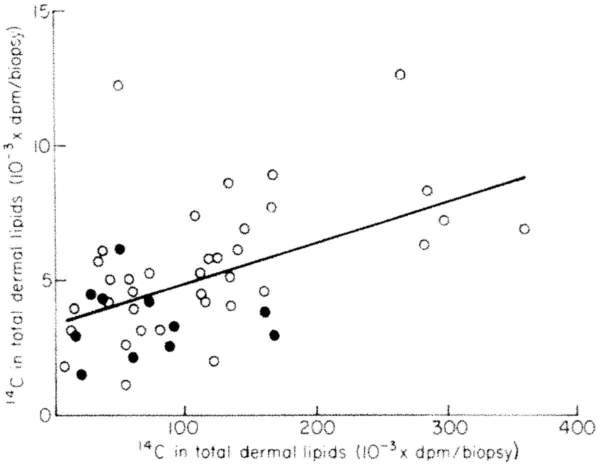

FIGURE 2. Correlation between epidermal and dermal lipogenesis in skin biopsies from subjects with mild acne (grades 0–1) (●) and severe acne (grades 2–4) (○). A regression line for all points is shown.

DISCUSSION

Our findings are that both epidermal and dermal lipogenesis are related to the severity of acne. The failure to find such a correlation in our previous study (Cooper *et al.*, 1976c) was as we suggested due to the much smaller group of patients studied. The possibility that the increased epidermal lipogenesis in severe acne is an artifact, caused by carry-over of sebaceous lipid, can be excluded because the characteristic epidermal lipid labelling pattern is maintained (Table 1). Whether the increase is general throughout the epidermis, or confined to the mouths of the pilosebaceous ducts, cannot be determined from the present data.

Although there is a significant correlation between dermal and epidermal lipogenesis, there is considerable individual variation; in some patients with severe acne increased epidermal lipogenesis appears to be associated with a normal rate of dermal lipogenesis. To some extent this may reflect sampling errors in the numbers of small and large sebaceous glands biopsied in a 4 mm disc; however, it may also be due to the greater destruction of sebaceous glands in severe acne than of the epithelium above them.

The keratinization and lipid deposits found in the sebaceous ducts in acne are generally believed to be the response to an irritative product of sebum produced by bacterial decomposition (Kligman, 1974). Although non-specific irritation does increase epidermal lipogenesis, it results in a dramatic shift in lipid pattern (Prottey & Hartop, 1973) quite unlike the change we have found.

The overall similarity in magnitude of the changes in dermal and epidermal lipogenesis and the general parallel between changes in lipogenesis and keratinization (Ziboh & Hsia, 1972; Cooper *et al.*, 1976c, 1978b; Cooper, McGibbon & Shuster, 1978a; Cooper, Wilson & Shuster, 1978c; Dykes & Marks, 1978) suggest the alternative possibility of a common control mechanism causing increased differentiation of sebaceous glands with lipogenesis and seborrhoea, and differentiation of the pilosebaceous ducts with lipogenesis and keratinization.

We suspect that the changes in the gland are the primary defect in adolescent acne (Cunliffe & Shuster, 1969; Burton & Shuster, 1971); the present findings will help to resolve the disputed role of the pilosebaceous duct if the inhibitors of sebaceous lipogenesis which ameliorate acne are found to have no effect on epidermal lipogenesis.

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Differences in the lipid constituents of sebum from pre-pubertal and pubertal subjects

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SUMMARY

Significant differences have been reported in the composition of skin surface lipid in pre-pubertal subjects when compared to pubertal subjects. Analytical studies were performed to determine whether group mean changes in the fatty acid composition of the triglyceride and wax ester fractions of sebum could be detected in pre-pubertal versus pubertal subjects. Twenty males (ages 6–9), twenty females (ages 6–9) and twelve teenagers (ages 11–16) were studied. Skin surface lipid was examined by densitometry and gas chromatography.

There were significant changes in the fatty acid composition of the wax fraction of sebum in the 11–16-year-old children when compared to the 6–9-year-old group. As wax is of sebaceous gland origin, this may represent a change in sebum composition probably in response to the hormonal stimulus. Changes in the fatty acid fraction of triglycerides were also noted with age, but this may be due to the change in source of triglyceride from predominantly epidermal origin to sebaceous gland origin.

Many investigators have made important contributions to our knowledge of the lipid constituents of sebum (Nicolaides, 1963; Kellum, 1967; Downing & Strauss, 1974). More detailed information on the influence of hormones on the lipid composition of sebum in the presence or absence of acne vulgaris is needed. This information should include analysis of quantitative compositional changes taking place in sebum, such as the fatty acid composition of triglycerides or wax esters as correlated with age, hormonal stimulation, and the presence or absence of acne. The quantitative studies by Ramasastry *et al.* (1970) on classes on lipids present in sebum as a function of age are an important step in this direction. There is evidence from animal studies that the fatty acid composition of the triglyceride and wax ester fractions of sebum from the preputial gland in the mouse changes in re-

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sponse to hormonal stimulation (Sansone *et al.*, 1969). In addition, there are changes in the relative quantities of the various lipid classes present. A similar effect might therefore be observed in human sebum.

With this in mind, the present study was undertaken to determine whether or not group mean differences could be found within the constituents of the various lipids present in the sebum of pre-pubertal subjects versus pubertal subjects.

MATERIALS AND METHODS

Subjects

The pre-pubertal and pubertal volunteers studied—twenty males (ages 6–9), twenty females (ages 6–9) and twelve subjects (ages 11–16)—were all school students. The younger children are participants in a longitudinal study, and the pubertal group was selected from volunteers who had very mild and not clinically significant acne and who were not being treated for dermatological problems.

Materials

Absorbent rayon balls were purchased from Johnson & Johnson. All solvents were Reagent or Spectrar grade. Silica gel G was purchased from Brinkman Instruments. Standards for thin layer chromatography (t.l.c.) and gas liquid chromatography (g.l.c.) were from Applied Science.

Methods

All glassware was prewashed with distilled chloroform/methanol (2:1). The rayon balls were prerinsed with distilled hexane, dried, and kept desiccated until use.

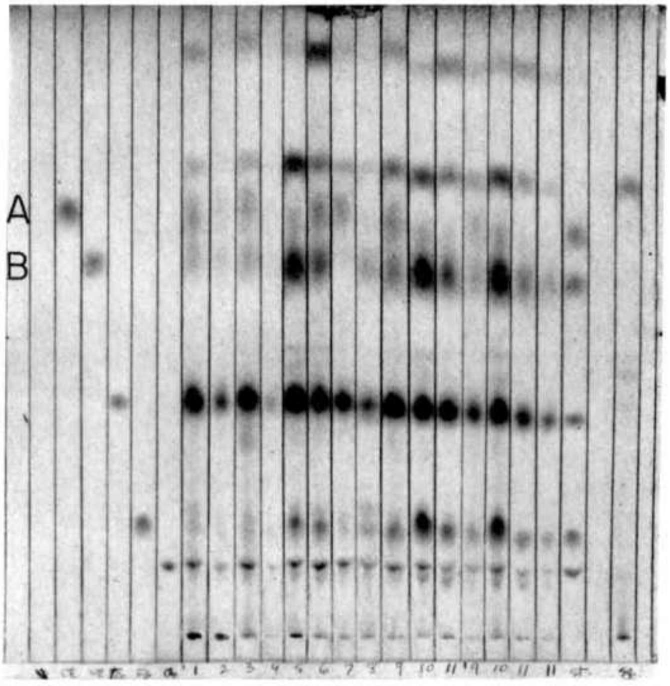

FIGURE 1. Thin-layer chromatogram showing separation of sterol esters (A) and wax esters (B).

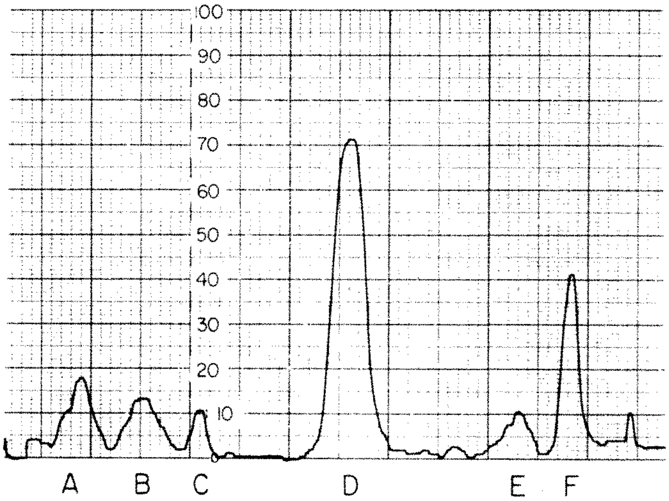

FIGURE 2. Densitometer tracing showing separation of sterol esters (B) and wax esters (C). A = squalene; D = triglyceride; E = free fatty acids; F = cholesterol.

Three lipid-free rayon balls were moistened with hexane and each serially used in wiping the face. The rayon balls were combined and thoroughly extracted with hexane through sintered glass funnels. The extract was evaporated to dryness and made to 1·0 ml with chloroform. An aliquot was then dried and lipids weighed on a Cahn balance.

Thin layer chromatography. Glass plates, 20 × 20 cm, were spread with 0·25 mm layer of silica gel G, heated at 110°C for 1 h, cooled, predeveloped in ether, and air-dried.

After spotting the plate, the multiple development system of Downing (1968) was used. The plate was first developed in hexane to the top of the plate, removed, air-dried for 10 min, followed by development in benzene to the top of the plate, removed, air dried for 10 min followed by development in hexane/ether/acetic acid (70:30:1) to the 10 cm level. The developed chromatograph was removed, air-dried, sprayed with 50% sulphuric acid, charred, and scanned on a densitometer.

Gas liquid chromatography. For g.l.c. analysis of the lipids in the various fractions, preparative plates 0·5 mm thick were used. Development was the same as described previously. The various lipid fractions were visualized with Rhodamine 6G. The wax ester and triglyceride fractions were scraped, eluted and separately rechromatographed. To assure purity, the wax ester fraction was saponified and the nonsaponifiable fraction was analysed by gas chromatography. Sterol content was less than 1%, indicating that the wax ester fraction had only trace contamination by sterol esters. Figure 1 shows the separation of wax esters and sterol esters on t.l.c. Figure 2 shows the densitometer tracing indicating clear separation of wax esters and sterol esters. The triglyceride and wax ester fractions were then transmethylated by scraping the respective bands into 5 ml ampoules, adding 2·0 ml methanolic-HCl, sealing and refluxing at 80°C for 2 h (triglycerides) and 4 h (wax ester fraction). The methyl esters were extracted with the addition of 1·0 ml water followed by two extractions with 1·0 ml hexane, back-washing the combined extractions once with 1·0 ml distilled water and drying with anhydrous sodium sulphate. The hexane extract was then evaporated under N_2 and made up to the desired volume. The wax ester fraction was further fractionated into two fractions: the fatty acid methyl esters (FAME) and the fatty alcohols. These were separated by t.l.c. using hexane/benzene (50:50), scraping

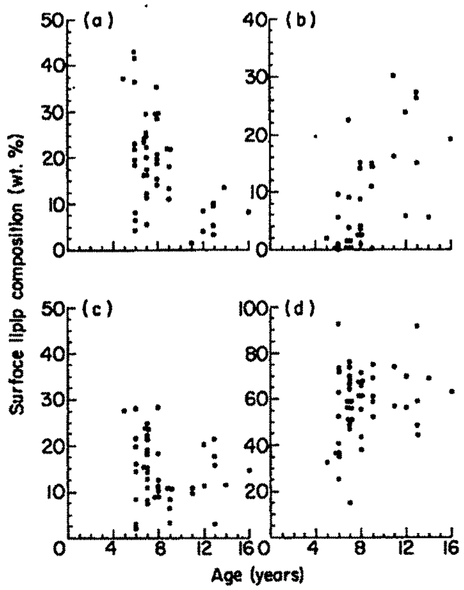

FIGURE 3. Variation in the composition of human skin surface lipid from pre- to post-puberty. For each sample of surface lipid, the concentrations of each of the four major constituents were plotted against the age of the subject. (a) Total cholesterol, $r = -0{\cdot}510$; (b) wax esters, $r = 0{\cdot}626$; (c) squalene; (d) glycerides + fatty acids.

the appropriate spot, elution, and analysis by g.l.c. The gas liquid chromatography of the FAME, of the triglyceride and wax fractions was performed following separation of the FAME by degree of unsaturation on thin layer chromatography plates of silica gel H 10% silver nitrate. Gas chromatography of the FAME was performed on a Varian Aerograph model 2100. The glass column, 6 ft × $\frac{1}{8}$ in, was packed with 10% EGSS-X on Chromosorb W (80–100 mesh). Carrier gas was nitrogen at a flow of 30 ml/min and column temperature of 175°C isothermal. Injection temperature was 180°C, flame ionization temperature was 185°C with H_2 at 30 ml/min and air at 300 ml/min. Temperature programming was also used. Conditions were: column—6 ft × $\frac{1}{8}$ in 10% SP-2330 on Chromosorb W (AW-DMCS 100–120 mesh). Temperature programmed from 140°C to 220°C at 6°C/min. Four minutes after injection. Injector temperature ≈ 210°C; detector temperature ≈ 240°C.

Two other columns were used to confirm the identity of the FAME samples: Apiezon L, 3% on Aeropack 30 (100–120 mesh), 10 ft × $\frac{1}{8}$ in (column 230°C, injector 245°C, detector 255°C); SE-30, 5% on DMCS Chrom W (60–70 mesh), 5 ft × $\frac{1}{8}$ in (column 180°C, injector 235°C, detector 245°C). The Apiezon L column was used to further confirm the saturates and unsaturates in the sample. The column reverses the elution pattern in that the unsaturates precede the saturated compound of the same carbon number. The SE-30 column is a general purpose column. This column separates compounds by molecular weight, altering the order of elution slightly in relation to the EGSS-X column.

RESULTS

In Fig. 3 are shown the variations in the composition of skin surface lipids with age. In the prepubertal group (ages 6–9) the preponderance of epidermal lipid is shown by the high content of cholesterol. As puberty ensues the proportion of wax esters rises significantly, indicating that the lipid is now predominantly of sebaceous gland origin. The concentrations of glycerides and fatty acids

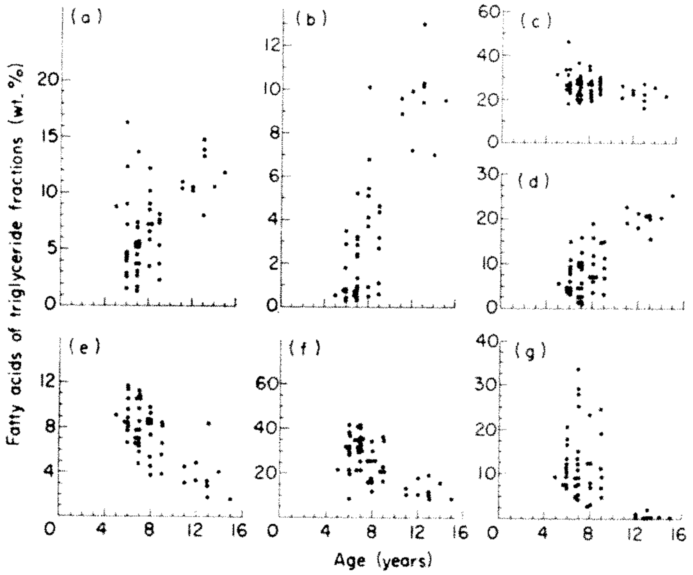

FIGURE 4. Variation in the concentration of fatty acids in the triglyceride fraction of human skin surface lipid. For each fatty acid, its percentage concentration (defined as wt. per cent of the total fatty acids of the triglycerides) is plotted against the age of the subject. (a) C_{14}, $r = 0{\cdot}511$; (b) C_{15}; (c) C_{16}, $r = -0{\cdot}331$; (d) $C_{16:1}$, $r = 0{\cdot}761$; (e) C_{18}, $r = -0{\cdot}691$; (f) $C_{18:1}$, $r = -0{\cdot}650$; (g) $_{18:2}$, $r = -0{\cdot}685$.

and squalene do not show similarly clear and significant trends. These findings are in agreement with those previously reported by Ramasastry *et al.* (1970).

The next step in our study was to separate the various lipid classes into their constituent parts, and analysis of the fatty acid methyl esters of the wax and triglyceride fractions was performed. The relative percentages of the various fatty acid methyl esters of the triglyceride fractions are shown in Fig. 4. There are significant changes in the relative concentrations of the major fatty acids constituting the triglyceride fraction. C_{15} levels rise from approximately 3% in the pre-pubertal group to 9% in the pubertal group. $C_{16:1}$ levels rise, while C_{18}, $C_{18:1}$ and $C_{18:2}$ sharply decline. These changes occurring in the composition of the surface lipid triglyceride fraction may represent a change from predominantly epidermal triglyceride to predominantly triglyceride of sebaceous gland origin (Downing & Strauss, 1974).

In Fig. 5 are shown the percentages of the various fatty acids in the wax ester fraction plotted against the age of the subjects.

There is a clear-cut decline in the percentage of C_{16} and a sharp decrease in the amount of $C_{18:1}$ in the wax ester fatty acids with age, while $C_{16:1}$ increases from approximately 20 to 40%. As it has been demonstrated that wax esters are of sebaceous gland origin, this represents a change in the composition of sebum.

DISCUSSION

The purpose of this study was to determine whether or not significant changes in the lipid composition of sebum could be found after hormonal stimulation of sebaceous glands during puberty. There were considerable changes in the fatty acid composition of triglycerides. It would not, however, be possible

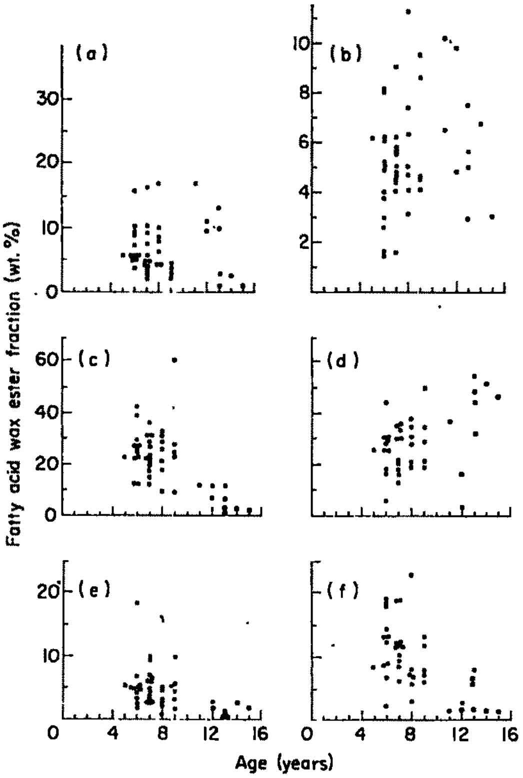

FIGURE 5. Variations in the concentration of fatty acids in the wax ester fraction of sebum. For each fatty acid, its percentage concentration (defined as wt. per cent of the total fatty acids of the wax fraction) is plotted against the age of the subject. (a) C_{14}; (b) C_{15}; (c) C_{16}; (d) $C_{16:1}$; (e) C_{18}; (f) $C_{18:1}$.

to determine the extent to which this could be attributed to changes in the major source of surface triglyceride from epidermal to sebaceous gland in origin, without more sophisticated and detailed studies. There is, however, a demonstrable difference in the fatty acid composition of the wax ester fraction. This change is probably in response to the alterations in hormonal status during and following puberty. Stimulation of the sebaceous glands to produce more sebum probably selectively shunts some of the fatty acid production into fatty alcohols, resulting in a different fatty acid pattern.

The ultimate goal of these preliminary analytical studies is to correlate qualitative and quantitative changes in sebum composition with changes in the hormonal environment of the sebaceous glands. This may help to define alterations in the character of sebum at puberty which might be critical in producing acne, such as changes that favour increased viscosity of sebum, increased irritancy, increased ability to promote the growth of bacteria or to provide alterations in the immune response or cause a hyperkeratotic response in the ductal epithelium.

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Sebaceous gland suppression in female acne patients by combined glucocorticoid-oestrogen therapy*

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SUMMARY

Women with severe acne were treated with a combination of oestrogen and a low dose of glucocorticoid daily in order to suppress their androgen production. This combination therapy reduced their sebum production by 50%, with a concomitant improvement in their acne.

In women with hirsutism, plasma androgens are suppressed by about 50% by daily combined treatment with oestrogen and a low dose of corticosteroid (Aakvaag, Vogt & Fylling, 1970; Casey, 1975). Since acne vulgaris is also provoked by androgenic stimulation of the pilo-sebaceous unit, and many women with acne have plasma androgen levels close to or above the upper limit of normal (Forstrom *et al.*, 1974), we decided to test the effect of such a regime on the sebum excretion rate in patients with acne.

MATERIALS AND METHODS

We studied nine males aged 17–25 years and six females aged 18–36 years all of whom had severe acne vulgaris. The female patients all had regular periods and no other evidence of virilization. They were treated for 4 months with prednisone 5 mg daily (taken in the late evening to produce the maximal adrenal suppression) and the oral contraceptive Minovlar (one tablet daily) for the first 21 days of each menstrual cycle. Each Minovlar tablet contains 50 μg of ethinyl oestradiol and 1 mg norethisterone acetate. This preparation was chosen because we had previously found that it suppresses sebum excretion more effectively than many of the other modern contraceptive pills (Pye *et al.*, 1977). The male patients were treated with 5 mg prednisone nocte and 30 μg ethinyl oestradiol daily. The lower dose of oestrogen was chosen to decrease the risk of gynaecomastia.

In every patient the sebum excretion rate was measured by a gravimetric method (Cunliffe &

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* This work was presented at the Summer B.A.D. Meeting in London, July 1979.

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Shuster, 1969) before treatment and after 8 and 16 weeks' treatment. The clinical state of the patient was also carefully assessed by the same doctor at each visit and was recorded as 'definitely better', 'possibly better', 'unchanged' or 'worse' relative to the initial visit.

RESULTS

The mean sebum excretion rates as a percentage of the pre-treatment value are shown in Figs 1 and 2 (the absolute values were distributed normally). The male patients showed no significant decrease during treatment, but the females showed a significant decrease at both 2 and 4 months, the rate at 4 months being about 50% of the initial value ($P<0.001$). All of the women showed a definite improvement in their acne at 4 months, and seven of the nine males also showed a definite improvement.

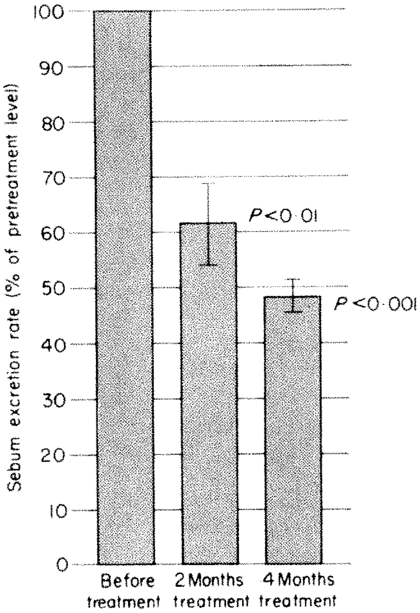

FIGURE 1. Sebum excretion rate in women with acne before and during treatment with prednisone 5 mg nocte and Minovlar for 21 days each month. Bar indicates mean ± s.e. mean.

DISCUSSION

In the female, androgens are produced by both the adrenal glands and the ovaries, and we have now shown that the use of combined low-dose prednisone and oestrogen therapy, which suppresses adrenal and ovarian androgen production, will reduce the sebum excretion in female acne patients by about 50%. The observed decrease was greater than we should have expected from the use of either prednisone alone or Minovlar alone. Even 20 mg prednisone daily suppresses the sebum excretion rate only to about 80% of the pre-treatment value (Pochi & Strauss, 1967), and Minovlar alone suppresses the rate to about 66% of the normal value (Pye *et al.*, 1977). In a similar study of hirsute females Pochi & Strauss (1976) found that 5 mg prednisone daily for periods ranging from 5 to 20 months reduced sebum excretion to 63% of the pre-treatment value, but the addition of ethinyl oestradiol 100 μg daily for 3 or more months caused a further dramatic fall to 14% of the

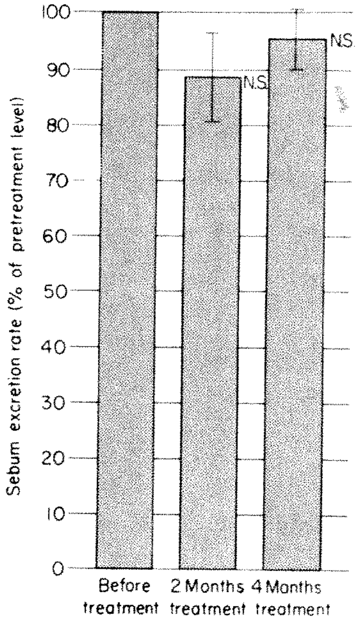

FIGURE 2. Sebum excretion rate in men with acne before and during treatment with prednisone 5 mg nocte and ethinyl oestradiol 30 μg daily. Bar indicates mean ± s.e.

original level. In the present study we used a smaller (and therefore safer) dose of oestrogen and we noted a less dramatic decrease in sebum production but there was nevertheless a marked improvement in the clinical state of every female patient treated.

The long-term safety of this regime in women with acne has yet to be assessed. The potential side-effects of oral contraceptives are well-known (R.C.G.P., 1974). There is very little risk of direct toxicity from 5 mg prednisone daily, but there is a small risk that after long-term treatment the suppressed adrenal gland may fail to respond to subsequent stress. This risk was assessed in thirty-nine patients with rheumatoid arthritis who had each taken 5 mg prednisone daily for periods of several years (Hicklin & Wills, 1968). Only four of these patients showed an abnormal response to a tetracosactrin (Synacthen) test, and only one patient showed an abnormal response to an insulin stress test. It seems likely therefore that a small percentage of patients would develop adrenal unresponsiveness during long-term treatment with the regime we used, and it would be wise for women receiving this treatment to carry a 'steroid' card, and to taper off the prednisone slowly (say 1 mg monthly) at the end of therapy. The regime is probably safe for short-term use in women with severe acne, and a controlled study is now required to compare its clinical efficacy with conventional therapy.

The lack of sebum suppression by combined prednisone–oestrogen therapy in the male was not unexpected, since testicular testosterone production is unaffected by the small doses we used, and Pocchi & Strauss (1973) have shown that in male subjects the sebaceous gland suppression produced by a much larger dose of ethinyl oestradiol (1 mg daily) can be completely reversed by the concomitant administration of a small dose of oral methyl testosterone (5 mg daily). The apparent improvement in the clinical status of the male acne patients despite the lack of sebum suppression was more surprising. This may be partly attributable to the placebo response, but we cannot exclude the possibility that the anti-inflammatory effect of the small dose of prednisone was responsible. The possible suppression of inflammatory papules of acne by small doses of steroid in the male requires further investigation, particularly in view of reports that immune mechanisms may be involved in the pathogenesis of inflammation in acne (Gowland *et al.*, 1978; Puhvel *et al.*, 1977).

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Use of biofeedback in treating chronic hyperhidrosis: a preliminary report

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SUMMARY

Preliminary findings attesting to the successful therapeutic use of biofeedback training in reducing symptoms of chronic hyperhidrosis are reported. Eleven of the fourteen adult patients trained with biofeedback were able to demonstrate clinical improvement in their excessive sweating 6 weeks after termination of treatment. Relaxation was suggested as the active ingredient in the biofeedback treatment effect. These findings support a recent report of the successful use of biofeedback in treating patients with dyshidrotic eczema.

Recent reviews by Bär & Kuypers (1973) and Masur (1977) indicate the potential therapeutic use of behaviour therapy techniques in treating dermatological disorders. To date, techniques such as systematic desensitization, progressive relaxation exercises, assertive training, and extinction (selective inattention to a patient's symptoms) have proved beneficial in alleviating disorders such as chronic constitutional eczema, atopic dermatitis, severe lichen simplex, urticaria, and hyperhidrosis.

Interestingly, biofeedback training, a behavioural technique frequently used in treating somatic disorders (Ray *et al.*, 1979), has received little attention in treating disorders of the skin. A recent report in this journal by Miller & Coger (1979), however, suggests that biofeedback may in fact be effective in treating patients with dyshidrotic eczema, both by producing marked changes in skin conductance and by lowering levels of subjective anxiety. In the latter study, clinical improvement was noted in sixteen of twenty-two eczema patients who were trained to decrease skin conductance via auditory biofeedback. Of those improved, 93% showed concomitant decreases in subjective anxiety. Similar improvement was not found for eleven patients who received biofeedback training for increased skin conductance (controls); these patients also showed signs of increased anxiety.

The present report describes the use of biofeedback training in the treatment of patients whose primary symptom was chronic excessive sweating—hyperhidrosis. The rationale for choosing biofeed-

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back as a treatment strategy was based on (a) the realization that chronic hyperhidrosis can result from anxiety and emotional stimuli (Bar & Kuypers, 1973); and (b) the suggestion that persons with hyperhidrosis can learn to decrease sweating with the help of behavioural-conditioning techniques (Kuypers & Cotton, 1972)*.

MATERIALS AND METHODS

A series of fourteen adult patients, ranging in age from 20 to 53 years, received biofeedback training for hyperhidrosis. All of the patients complained of excessive, troublesome sweating over an extended period of time. All had been referred to the dermatology clinic of a large European medical school for diagnosis and treatment of their sweating disorder. Eight of the patients were hospitalized at the time of biofeedback treatment; the remaining six were seen as out-patients. Eight of the patients were male; six were female. One of the patients was diagnosed as having contact eczema; two others were diagnosed as having dyshidrotic eczema.

Biofeedback training was aimed at teaching patients to reduce the magnitude of their sweat response, as measured by the Meeco water vapour analyser (for details see Kuypers & Cotton, 1972). Excessive sweating was produced in patients both by discussing emotionally-laden topics with them and by mental arithmetic (Allen, Armstrong, & Roddie, 1973). Patients were able to monitor visually their progress by watching their sweat response on a mV recorder, where they were given both *directional* (with increased sweating the recorder pen moved to the right; with decreased sweating the pen moved to the left) and *quantitative* (patients were given a rating of the magnitude of their sweat response on a scale of 0–100) feedback by the therapist.

All biofeedback training sessions took place in the therapist's office and lasted for 30 min each. Hospitalized patients were seen twice a day for biofeedback, while out-patients were seen twice each week. While undergoing biofeedback training, all patients were removed from medication for hyperhidrosis; some patients continued to take medication for other non-dermatological disorders. Each patient received an unspecified number of biofeedback training sessions until some improvement was noted; treatment was terminated in patients who failed to show any ability to reduce the magnitude of their sweat response after ten training sessions. All of the patients were formally evaluated, 6 weeks after termination of biofeedback training, for signs of clinical improvement.

RESULTS

Patients were classified as clinically 'improved' or 'not improved' both in terms of (a) their own self-report statements about excessive, troublesome sweating outside of treatment and (b) the clinical observation of excessive sweating made by the therapist at the point of follow-up. Table 1 shows the overall results of biofeedback training based on these two criteria.

Eight of the fourteen patients not only reported significant improvement in their sweating condition as a result of treatment; they also provided clear evidence of such improvement by remaining dry during the follow-up interview. Two other patients appeared to sweat much less during the interview 6 weeks after treatment, but reported little improvement outside the clinic. One patient similarly reported that his sweating was less troublesome after treatment; yet he was observed to sweat excessively during the interview with the therapist. The three remaining patients showed no improvement using either criteria of symptom change; these were patients who were unable to show improvement after ten sessions and thus had their treatment terminated early on.

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* The Miller & Coger (1979) findings were not published at the time the present study was formulated and thus did not provide a basis for deciding that biofeedback was a suitable treatment for patients with hyperhidrosis. The similarity in approach and outcome in the two studies is serendipitous.

TABLE 1. Effect of biofeedback in patients with hyperhidrosis

Patients	Sex	Age (years)	In-patient or out-patient	Location of sweating	Outcome	
					Self-report	Observation
1	M	27	In	Hands, feet, armpits	+	+
2	F	28	Out	Hands, feet, armpits	−	+
3	M	33	Out	General	+	−
4	M	20	In	Hands	+	+
5	M	53	Out	Forehead	+	+
6	M	24	Out	General	+	+
7	F	23	In	Hands, feet	+	+
8	M	31	In	Hands	−	−
9	F	20	Out	Hands, feet, armpits	+	+
10	F	33	Out	Hands, feet, armpits	−	−
11	F	22	In	Hands, feet	+	+
12	M	34	In	Hands	+	+
13	F	28	In	General	−	−
14	M	26	In	General	+	−

+ Indicates the patient was 'improved'; − indicates he was 'not improved'.

Overall, eleven of the fourteen hyperhidrosis patients showed clinical improvement as a result of biofeedback training, based on the therapist's observation or their own subjective report. None of the patients dropped out of treatment and all of those who could learn to reduce their sweat response via visual biofeedback within the first ten training sessions showed signs of clinical improvement 6 weeks after treatment was ended.

DISCUSSION

These results, while preliminary, do suggest the possibility that biofeedback training may be effective in treating patients with chronic hyperhidrosis. They support the positive findings of Miller & Coger (1979), using biofeedback with dyshidrotic eczema patients. They suggest that visual and verbal (therapist) feedback are effective in this type of treatment; Miller & Coger had used auditory (tone) feedback. Our results also suggest the successful use of biofeedback in the clinic; whereas Miller & Coger had patients practise biofeedback on their own in their home environment.

Perhaps most important, these findings, along with those of Miller & Coger, support the idea that relaxation may be conceived as the 'final common pathway' in the treatment of many psychophysiological disorders (Silver & Blanchard, 1978) and that the active ingredient of biofeedback is relaxation or tension-reduction (Ray *et al.*, 1979). When asked about how they were able to reduce their sweat response, the hyperhidrosis patients generally reported that they 'kept silent' and 'relaxed'. This increased ability to relax most probably accounted for the significant decline in subjective anxiety noted for improved eczema patients in the Miller & Coger (1979) study.

It is also important to note that not all patients show clinical improvement in dermatological disorders when they receive biofeedback training. As in the Miller & Coger (1979) study, some of our patients could not effectively demonstrate a decrease in the physiological behaviour (sweat response) underlying their skin disorder. Why they could not effect a change in their sweat response is not clear. The fact that three of the four patients who did not show clear evidence of improvement during the interview were females may be important; also, it was noteworthy that three of the five patients who continued to complain of excessive, troublesome sweating were patients suffering from 'generalized' hyperhidrosis, i.e. sweating over large areas of the body, as compared to patients with sweating problems in specific areas, e.g. forehead or hands. The fact that Miller & Coger found no differences in clinical improvement in terms of area of involvement suggests the need for more careful study of individual differences in treatment outcome when using biofeedback with dermatology patients.

Using the model offered by Ray *et al.* (1979), it is clear that the ultimate test of clinical utility for biofeedback in treating this and other types of skin disorders will depend on further investigations which look at: (a) the effect of biofeedback in relation to no-treatment control conditions; (b) the issue of cost-effectiveness (i.e. if relaxation proves to be the active ingredient in such treatment, a simpler form of relaxation training may prove more useful); (c) non-specific factors and placebo effects; and (d) an evaluation of objective (physiological) changes in comparison to subjective (psychological) outcome. At this point, these results and those of Miller & Coger (1979) would suggest that biofeedback training can only be as successful in the treatment of dermatological disorders as it has thus far proved to be for such disorders as: musculoskeletal pain, insomnia, spasmodic torticollis, gastro-intestinal dysfunction, and cardiac disorders such as premature ventricular contractions and Raynaud's disease.

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Pagetoid reticulosis: a disease of histiocytic origin

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SUMMARY

A patient with Pagetoid reticulosis has been investigated using standard transmission electron microscopy and an immunohistochemical technique for identifying T lymphocytes *in situ* in light and electron microscopy. Our results indicate that the majority of the cells in the underlying dermal infiltrate are T lymphocytes but that only very few T lymphocytes are found in the extensive epidermal infiltrate. Ultrastructurally the majority of the abnormal cells in the epidermis are of histiocytic origin.

In 1939 Woringer & Kolopp reported the case of a 13-year-old patient with a 6-year history of an isolated erythemato-squamous lesion on his forearm. Histological examination of the lesion showed marked infiltration of the epidermis by abnormal cells and an underlying inflammatory dermal infiltrate. The exact nature of the disease remained unclear but on histological grounds the authors entertained diagnoses of a cellular naevus, extramammary Paget's disease and mycosis fungoides but found clinical or pathological objections to all these diagnoses. The authors also suggested the possibility of a neoplasm of the Merkel-Ranvier cells, but could not substantiate this.

Since the original description, eighteen case reports of Woringer–Kolopp disease or Pagetoid reticulosis have appeared in the literature. Eight of these have shown isolated lesions similar to that described by Woringer and Kolopp (Braun-Falco *et al.*, 1979), but ten have been of patients with disseminated skin disease clinically resembling mycosis fungoides but with the histological appearances of the Woringer–Kolopp lesion (Braun-Falco *et al.*, 1979).

Many of the authors support the suggestion that Pagetoid reticulosis is a particularly epidermotropic variety of mycosis fungoides (Castermans-Elias, 1974; Degreef *et al.*, 1976; Dupré *et al.*, 1977; Haneke, Tulusan & Weidner, 1977; Lever, 1977; Burg *et al.*, 1977). This is partly due to the clinical similarity between the disseminated Ketron Goodman variant (Ketron & Goodman, 1931) of Pagetoid reticulosis and mycosis fungoides, and the occurrence of parapsoriasis (Dupré *et al.*, 1977), parakeratosis variegata (Haneke *et al.*, 1977) and even mycosis fungoides (Degreef *et al.*, 1976) in association with Pagetoid reticulosis. Also, there are obvious similarities between the light microscopic appearance of Pagetoid reticulosis and mycosis fungoides (Lever, 1977).

In our study, in addition to conventional electron microscopy, we used an indirect immunoperoxidase technique employing a specific antiserum against the human T-lymphocyte differentiation

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antigen, HTLA, to identify T lymphocytes *in situ* in light and electron microscopy. Using this technique we were unable to characterize the abnormal cells in the epidermis as T lymphocytes, and ultrastructurally the abnormal cells possessed features that were more consistent with a histiocytic origin than a lymphocyte origin.

CASE HISTORY

F.W. (male) was born in 1919 and first noticed a lesion on the palmar aspect of his right hand following a thermal burn in 1945. The lesion was scaly and erythematous but asymptomatic. It has increased in size since that time but no further lesions have appeared. Treatment has comprised 1% hydrocortisone cream with 10% urea and various concentrations of salicylic acid ointment which have reduced the scaling.

Clinical examination revealed a 4×6 cm erythematous plaque overlying the right thenar eminence extending up to the interphalangeal joint of the thumb and onto the dorsum of the hand.

MATERIALS AND METHODS

Anti HTLA (Institut Merieux) was produced in rabbits and its specificity controlled by microlymphocytotoxicity, rosette inhibition and ultrastructural enzymatic labelling techniques. It was used at a dilution of 1:2 in phosphate buffered saline (PBS).

Sheep anti-rabbit Fab Po (Institut Pasteur) was used in a dilution of 1:30 in PBS.

Sheep anti-mouse Fab Po (Institut Pasteur) was used as a negative control serum at a dilution of 1:30 in PBS.

Embedding resin—TAAB (TAAB Laboratories Reading).

Four biopsies were taken from the lesion under local anaesthesia. Each was processed in a different way:

(1) Fixed in 4% formol saline, paraffin embedded and sections stained with haematoxylin and eosin for light microscopic examination.

(2) This was cut into 1–2 mm cubes and fixed for 2 h in 2% glutaraldehyde. Following an overnight wash in cacodylate buffer the cubes were post-fixed in 1% osmium tetroxide for 2 h, dehydrated in graduated alcohols and resin embedded. Semi-thin 1 μm sections were examined counterstained with Richardson's stain (Richardson, Jarett & Finke, 1960), and ultra-thin sections were stained with lead citrate and uranyl acetate before examination at 75 kV in a Hitachi HU 12A electron microscope.

(3) This was snap frozen in N-hexane and carbon dioxde (Chayen *et al.*, 1969) and stored at −70°C.

(4) This was cut into 1–2 mm cubes and fixed for 2 h in 3% paraformaldehyde containing 8·5% sucrose. After an overnight wash in PBS containing 8·5% sucrose, the cubes were washed in distilled water, snap frozen in N-hexane and carbon dioxide, and then stored at −70°C (Chu, Morgan & MacDonald, 1980).

Using the fresh frozen material (3) 4 μm cryostat sections were cut onto glass slides and were fixed for 10 min in 2% paraformaldehyde. Cryostat sections (15 μm) of the prefixed frozen material (4) were cut onto glass slides previously treated with egg albumen.

After a careful wash in PBS, the 4 and 15 μm sections were incubated for 30 min with the rabbit anti HTLA. The sections were then washed twice in PBS before incubation with the sheep anti-rabbit Fab Po or control serum. After sequential washing in PBS and tris HCL the peroxidase activity was revealed histochemically using 3′3′ diaminobenzidine and hydrogen peroxide.

At this stage the 4 μm sections were washed in PBS, dehydrated through graduated alcohols, bathed in xylene and mounted in DPX for examination in a Zeiss photomicroscope.

The 15 μm sections were post fixed in 1% osmium tetroxide for 10 min, dehydrated, impregnanted with resin and while still on the glass slides were included in resin blocks by inverting resin filled gelatin capsules over the sections and polymerizing at 60°C for 16 h. The blocks were separated from the glass slides by immersion in liquid nitrogen and the blocks trimmed and cut on a Reichert Ultracut.

Sections, 1 μm thick, were examined, stained with Richardson's stain and areas selected for ultra-thin sectioning. Ultra-thin sections were collected on copper grids and half were examined unstained at 50 kV in a Hitachi HU 12A electron microscope and half were counterstained with lead citrate and uranyl acetate and examined at 75 kV.

RESULTS

Light microscopy

Histologically there was marked hyperkeratosis and parakeratosis with considerable epidermal acanthosis and papillomatosis. In haematoxylin and eosin (H & E) stained sections the most remark-

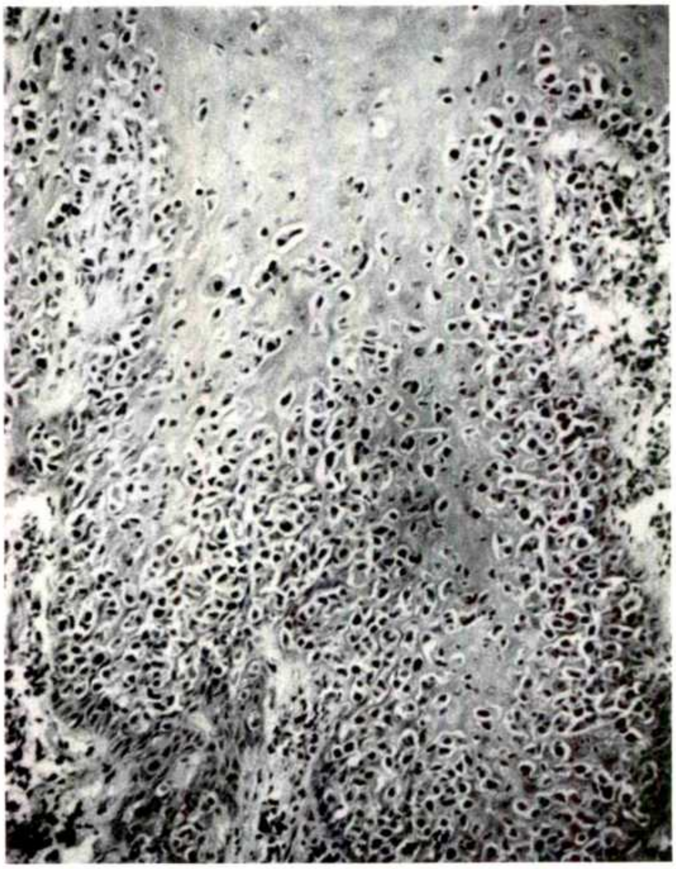

FIGURE 1. Pagetoid reticulosis. Infiltration of epidermis by large mononuclear cells. The majority of the dermal infiltrate consists of mature lymphocytes (H & E, × 160).

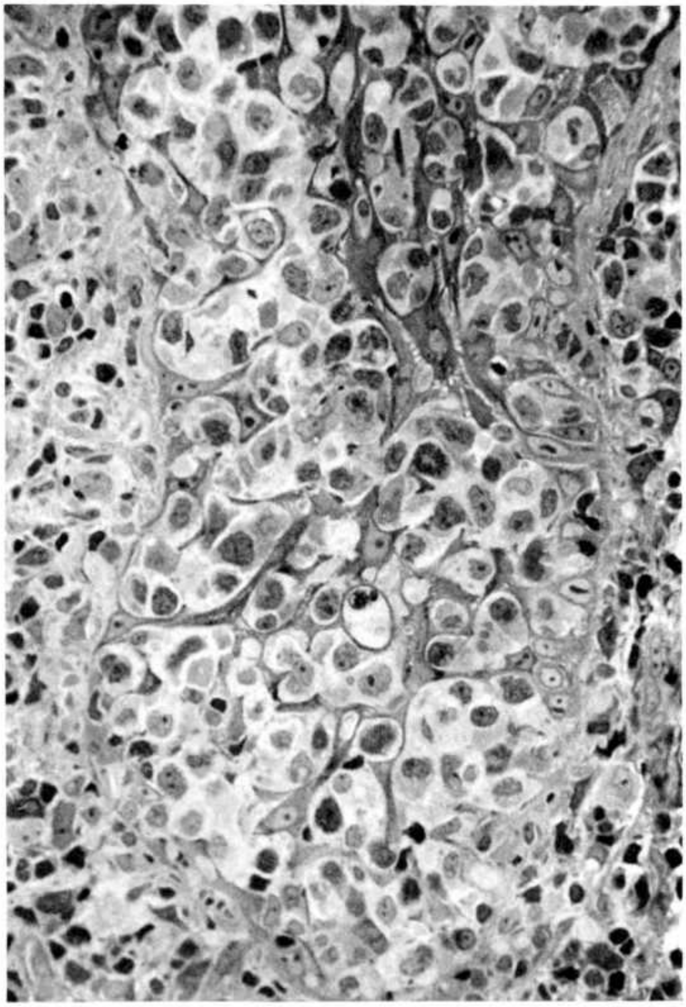

FIGURE 2. Pagetoid reticulosis. Resin embedded semi-thin sections (Richardson's). Epidermal infiltrating cells possess highly convoluted nuclei with prominent nucleoli. The apparent clear halo around individual cells represents pale staining cytoplasm. Mitotic figures can be seen (× 520).

able feature was infiltration of the epidermis, particularly the lower layers, by extremely large mononuclear cells with hyperchromatic nuclei and a high nuclear/cytoplasmic ratio. The nuclei were of variable shape, some being round or oval while others were angulated or convoluted. Mitotic figures, often of a bizarre nature, were frequent. Individual infiltrating cells were apparently separated from each other and from adjacent keratinocytes by a clear halo. The papillary and upper reticular dermis also showed infiltration by dense collections of mononuclear cells. Small foci of cells similar to those in the epidermis were present but the majority of the dermal infiltrate consisted of mature lymphocytes whose cell size was one-third or less of that of the cells infiltrating the epidermis. Plasma cells, mast cells and normal histiocytes were also prominent in the dermal infiltrate (Fig. 1).

Nuclear detail of the epidermal infiltrating cells was more easily studied in resin embedded semi-thin sections. The features described above were confirmed but in addition it was evident that nuclear

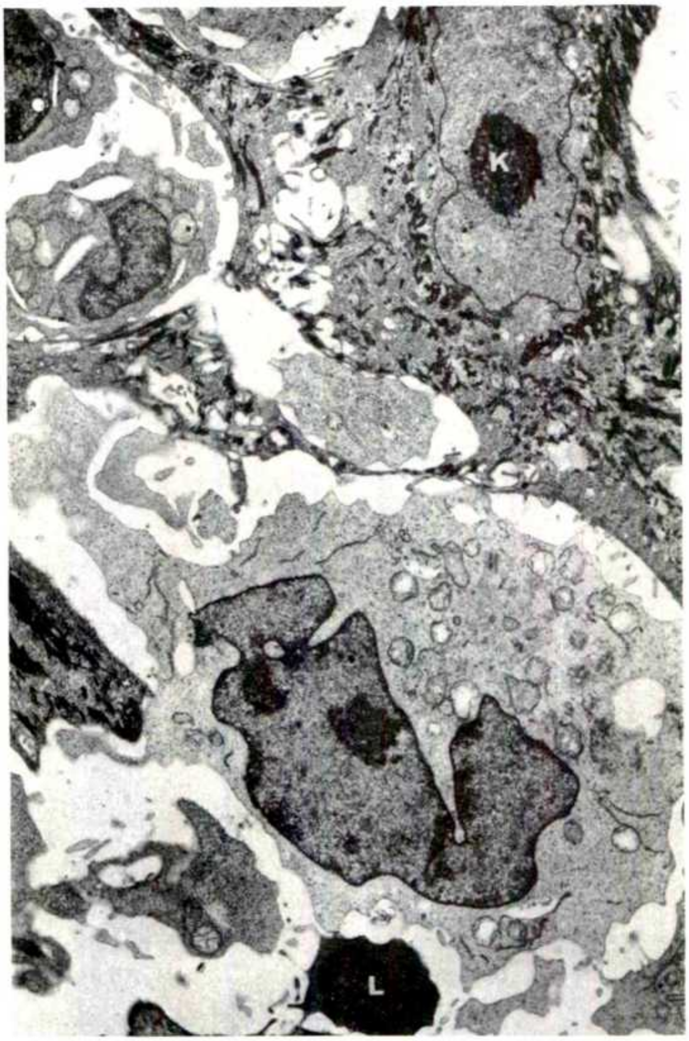

FIGURE 3. Mononuclear cell infiltrating the epidermis possessing abundant cytoplasm containing numerous organelles. Nucleus is indented into geometric shapes and the chromatin is condensed at the periphery. L = normal lymphocyte; K = keratinocyte (× 4800).

outlines were frequently highly convoluted, prominent nucleoli were frequently visible, heterochromatin/euchromatin ratios were variable with the heterochromatin situated just beneath the nuclear membrane. The apparent clear halo around individual cells comprised pale staining cytoplasm. The cells resembled in some respects 'mycosis' cells but the morphological details were more suggestive of a histiocytic origin (Fig. 2).

Ultrastructure

In the epidermis, the keratinocytes appeared normal and occasional normal melanocytes and Langerhans cells were identified. Numerous abnormal cells, however, were seen infiltrating between the

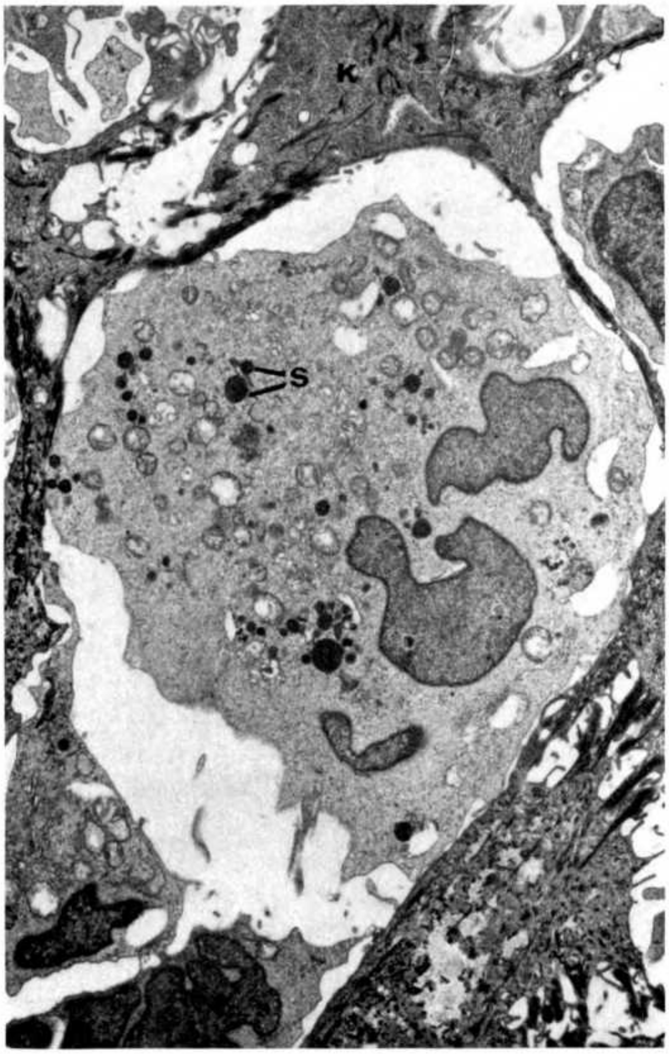

FIGURE 4. Mononuclear cells infiltrating the epidermis. S = secondary lysosome; K = keratinocyte (× 4800).

keratinocytes. A few lymphoid cells were present in this infiltrate, most of which appeared to be normal lymphocytes, but very occasionally lymphoid cells with deeply indented nuclei and scanty cytoplasm were observed.

The majority of the infiltrating cells present in the epidermis were large cells with abundant cytoplasm. The nuclei tended to be indented into geometric shapes and occasionally the plane of sectioning led to the appearance of intranuclear inclusions. The chromatin was condensed at the periphery of the nuclei and some of the cells possessed small nucleoli. In the cytoplasm numerous mitochondria and vesicles were present and filaments were seen in many of the cells (Fig. 3). Ribosomes and polysomes were abundantly present free in the cytoplasm, and rough endoplasmic reticulum was occasionally identified. Occasionally, irregular electron dense bodies were present within the cytoplasm of the

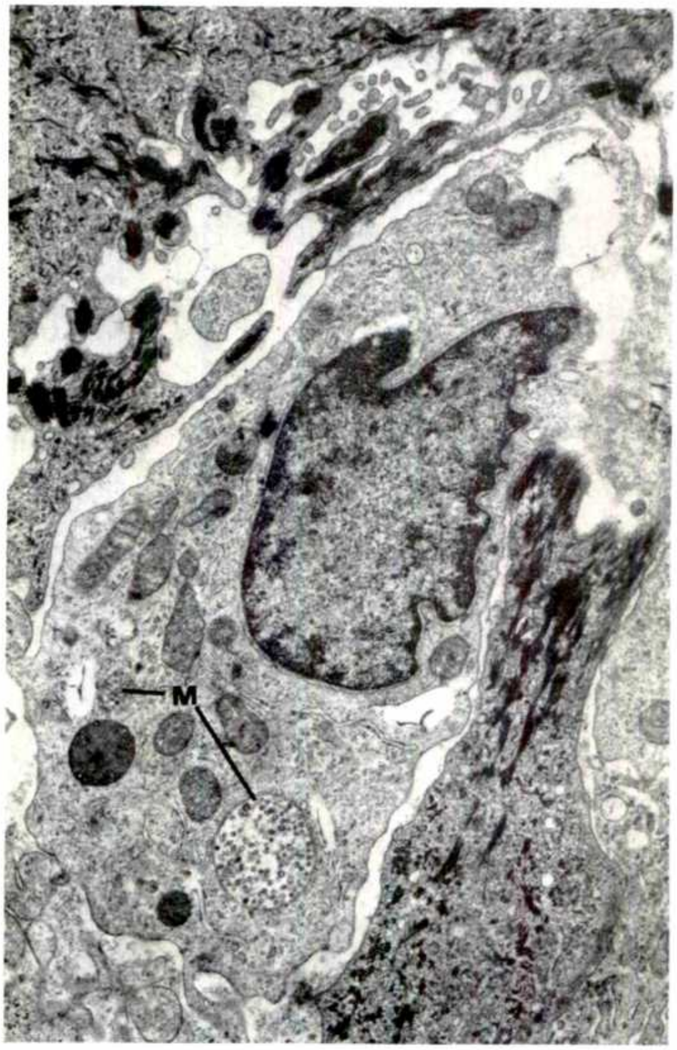

FIGURE 5. Pagetoid reticulosis. Infiltrating cell in the epidermis showing microvesicular bodies (M) (× 12,000).

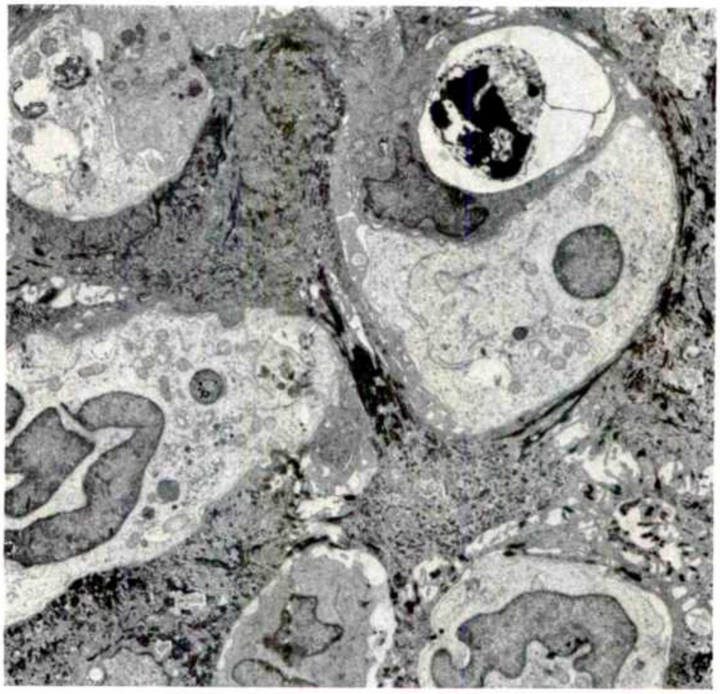

FIGURE 6. Shrunken and pyknotic cell present in the upper epidermis (× 3600).

cells which appeared to be secondary lysosomes (Fig. 4). A prominent feature of many of these cells was the presence of microvesicular bodies often intimately related to the Golgi apparatus (Fig. 5). The cytoplasmic membrane was thrown up into multiple folds and microvilli. Occasional shrunken and pyknotic cells were present lying free or in phagocytic vesicles in abnormal cells in the upper epidermis (Fig. 6). Cells in mitosis were also seen.

In the dermis, there was a mixed cellular infiltrate in which lymphoid cells predominated. Mast cells, eosinophils and macrophages were also identified. The lymphoid cells were generally mature lymphocytes but occasionally cells possessing deeply indented Sézary-like nuclei were present. Also present in the dermal infiltrate were groups of the abnormal cells seen in the epidermis.

Immunohistochemical studies

Light microscopically T lymphocytes could easily be identified by the presence of a fine brown granular deposit on the cells' surface. The majority of the cells in the dermal infiltrate were T lymphocytes but only very few T lymphocytes were seen in the epidermal infiltrate (Fig. 7).

Immunoelectron microscopically T lymphocytes were identified by the presence of the electron dense peroxidase reaction product which was seen as an irregular though continuous deposit restricted to the cytoplasmic membrane of the cells (Fig. 7a). The ultrastructural findings confirmed that most of the cells in the dermal infiltrate were T lymphocytes and that very few T lymphocytes were present in the epidermis. The counterstained sections showed that the labelled cells were morphologically mature T lymphocytes. The abnormal cells showed no positive specific labelling.

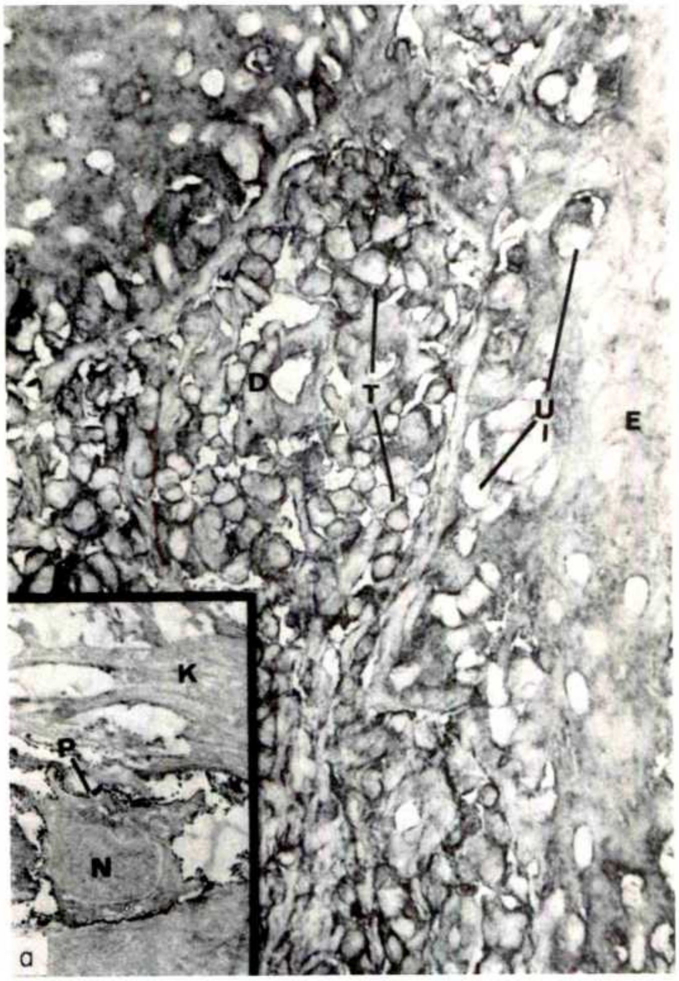

FIGURE 7. Cells in the dermal infiltrate showing positive T-cell labelling. Epidermal infiltrating cells show no labelling. T = positive T lymphocyte labelling; E = epidermis; D = dermis; U = unlabelled infiltrating cell (× 520).

(a) Isolated T lymphocyte present in epidermis. P = continuous deposit of peroxidase reaction product restricted to the cytoplasmic membrane; N = nucleus; K = keratinocyte (× 1600).

DISCUSSION

The exact nature of the pathological process involved in Pagetoid reticulosis has engendered considerable discussion and controversy. The cell type involved in the disease has never been precisely characterized and this has led to the confusion that surrounds Pagetoid reticulosis.

In 1939 Woringer & Kolopp suggested that their first case may have been a proliferation of the Merkel Ranvier cells. Revuz *et al.* (1971) revived this idea because of their findings of cytoplasmic granules with round electron dense cores within the abnormal cells which bore a close relationship to neurites. In our study we were unable to identify neural components within the abnormal epidermis and although some of the cells in the epidermis and dermis contained dense cytoplasmic granules similar to those described by Revuz *et al.* (1971), we interpreted these as being lysosomal vesicles and the cells to be of the histiocyte series.

Many reports in recent years have suggested on the basis of clinical, histological and ultrastructural studies that Pagetoid reticulosis is a variant of mycosis fungoides and that the abnormal cells in the epidermis are of lymphocytic origin. Our case shows similar ultrastructural characteristics to those described by Braun-Falco *et al.* (1979), Haneke *et al.* (1977), Degreef *et al.* (1976) and Medenica & Lorincz (1978), but we disagree with the interpretation of all but the first authors that these abnormal cells are lymphoid. Several facts lend support to our proposal that these cells are of histiocytic origin. The typical condensation of nuclear chromatin at the periphery of the nuclei and the rather geometric angulation of the nuclei often leading to the appearance of intranuclear inclusions is strongly suggestive of a histiocyte origin. The abundance of cytoplasm containing multiple organelles and in some instances phagocytic vesicles makes a lymphoid origin less likely. The presence of multivesicular bodies, which we found to be a prominent feature of these cells, is regarded as a histiocytic feature. Also the exaggerated folds of the cell surface were suggestive of a histiocytic origin.

Braun-Falco *et al.* (1979) in their study of a patient with Pagetoid reticulosis interpreted the ultrastructural findings as being more suggestive of a monocytic, promonocytic origin than a lymphocytic origin. In immunohistochemical studies, however, Braun-Falco *et al.* (1979) substantiated the previous reports of Burg *et al.* (1977) that most of the cells in the cutaneous infiltrate were T lymphocytes. The methods used by Burg *et al.* (1977) were the use of spontaneous rosette formation and of an anti T-cell globulin on suspensions of cells extracted from the skin. Although histologically the epidermal infiltrate appears striking and very extensive, the actual number of abnormal cells present in the epidermis is small compared to cell numbers comprising the dermal infiltrate. Also, ultrastructurally the cells in the epidermal infiltrate were seen to have microvillous cell surfaces, and it is quite possible that these cells would be damaged on extraction from the skin or would be more difficult to extract than the cells in the dermal infiltrate. The possibility remains, therefore, that the cell population examined was not representative of the epidermal infiltrate. Braun-Falco *et al.* (1979) also used a peroxidase anti-peroxidase technique with an anti T-cell globulin on cryostat sections of skin and showed that 70% of cells present in the cutaneous infiltrate were T lymphocytes, but did not discuss the precise localization of the cells within the tissue.

In our study, an *in situ* technique was used in light and electron microscopy and the majority of the cells in the epidermal infiltrate were found not to possess the human T lymphocyte differentiation antigen HTLA. This is totally different from the results of our previous studies on mycosis fungoides where the majority of the cells in the epidermal infiltrate were found to be T lymphocytes (Chu & MacDonald, 1979; Chu *et al.*, 1980).

Compared with other reports of Pagetoid reticulosis our case appears identical in clinical, histological and ultrastructural features. Our findings, however, suggest that this disease, rather than being a T-cell lymphoma of low degree malignancy, is in fact a disease of histiocytic origin.

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B lymphocytes in contact dermatitis

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SUMMARY

The expression of immunoglobulin on the membrane of peripheral blood lymphocytes of patients with contact dermatitis has been investigated using a sensitive rosette technique. The proportion of such cells in these patients is increased compared to normal values. However, the absolute values obtained are very much lower than has been reported from previous studies. The re-evaluation of immunoglobulin expression is discussed against a background of improved methodology and the established finding that individual lymphocytes may express more than one isotype.

Several studies have demonstrated alterations in the relative frequency of B and T lymphocytes in the peripheral blood of patients with eczematous skin reactions and also abnormalities in the proportions of B cells bearing the various immunoglobulin heavy chain determinants (Cormane, Hamerlink & Husz, 1975; Carpeto, Winkelmann & Jordon, 1976; Kermani-Arab, Roberts, & Leslie, 1978). In a study of patients with contact dermatitis Cormane *et al.* (1975) reported that Ig-bearing cells accounted for 20–87% of peripheral blood lymphocytes and that the increase over normal values was due to the presence of a population of IgD bearing cells. From a similar study Carpeto *et al.* (1976) reported values of 16–48% for total peripheral B cells and 3–23% for IgD bearing cells. In each of these studies the normal values reported for peripheral B lymphocytes were greater than the values now generally accepted.

A revision downwards in the value of Ig positive cells detected in normal peripheral blood has resulted from a recognition of the fact that some B cells and monocytes bear receptors for the Fc region of IgG (human and rabbit) and thus bind this immunoglobulin class cytophilically (Lobo, Westervelt & Horwitz, 1975). Techniques currently in use are directed towards minimizing the interference of such cytophilically bound IgG in the detection system. Also, it is well established that an individual lymphocyte may express more than one heavy chain class (isotype) on its membrane (Kunkel, 1976) and thus the total immunoglobulin bearing lymphocyte population is not simply the sum of percentages of cells bearing each isotype.

An aspect of the studies of Cormane *et al.* (1975) and Carpeto *et al.* (1976) of particular interest to

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TABLE 1. Clinical data of patients with contact dermatitis

			Atopy				
Name	Sex	Age	Patient	Family history	Dermatitis type	Patch	Tests
SB	F	45	–	+	Endogenous contact	Nickel	(1+)
AL	F	31	–	–	Contact	Nickel	(2+)
MD	F	31	–	–	Contact	Nickel	(2+)
JS	F	18	–	–	Contact	Nickel	(2+)
JR	F	18	–	–	Contact	Nickel	(2+)
JH	F	33	–	+	Contact	Nickel	(1+)
JK (Indian)	F	28	–	–	Contact	Nickel	(2+)
KJ	F	18	–	–	Contact	Nickel	(2+)
LH	M	44	–	–	Contact	Nickel Chromate	(2+) (2+)
GA	M	52	–	–	Endogenous contact	Neomycin	(2+)
AJ	M	37	–	+	?Endogenous contact	Chromate	(1+)
MN	M	33	–	+	Endogenous contact	Colophony	(2+)

– = no atopy; + = atopy.
1+ = positive reaction; 2+ = strongly positive reaction.

us was the finding that the increase in B lymphocytes observed in patients with contact dermatitis was due to a predominant population of cells expressing IgD only—a cell type rarely observed in normal or other pathological states. The intriguing possibility that such a cell type might exist and be functional in this disease led us to re-evaluate the claim using the improved reagents and more sensitive techniques currently available.

MATERIALS AND METHODS

Clinical

The clinical data for patients with contact dermatitis are presented in Table 1. Diagnosis was based on the features of a positive patch test and the lack of atopy was further supported by the patient's previous medical history and the lack of atopy amongst family members.

Estimation of the percentages of lymphocytes bearing immunoglobulin of the different classes

Heparinized blood (20 ml) was treated with 2% dextran 500 in phosphate-buffered saline (4 ml) and allowed to sediment at 37°C for 1 h. The leukocyte rich supernatant was removed and centrifuged. The cell pellet was resuspended in Hepes-buffered RPMI 1640 medium containing 2% fetal calf serum, and the mononuclear cells separated by centrifugation through Ficoll-Triosil (Böyum, 1976). Separated cells were washed, resuspended in warm medium and incubated for at least 1 h at 37°C to remove cytophilically bound immunoglobulin after which the cells were again washed in warm medium and resuspended to 2×10^6/ml.

Samples of the cells were tested by a rosette test using bovine red cells coated with the specific sheep antibodies to the appropriate heavy or light chain exactly as described by Dhaliwal *et al.* (1978). A control with bovine red cells coated with serum immunoglobulin of an unimmunized sheep was always included (NSS in Table 2). Monocytes present in the mononuclear cell fraction were identified by their capacity to ingest opsonized dyed yeasts (Shaala *et al.*, 1979).

Serum immunoglobulins

Immunoglobulins G, M and A were quantified using an automated immune precipitation methodology incorporating a Technicon sampler, proportioning pump and manifold with a Vitatron nephelometer. A normal serum pool, calibrated against a Technicon reference serum, was used as a standard.

IgD was estimated by the single radial immunodiffusion technique using purified paraprotein as standard.

Antisera were kindly supplied by the Immunodiagnostic Research Laboratory, Department of Immunology, University of Birmingham, and are available through Seward Immunostics, UAC House, London SE1 9UG.

RESULTS

The percentage of B cells expressing a given immunoglobulin class was determined for lymphocyte preparations obtained from peripheral blood of twelve patients with diagnosed contact dermatitis

TABLE 2. Immunoglobulins on the surface of lymphocytes of patients and normals

	Percentage of rosettes								
	NSS	IgD	IgM	IgG	IgA	IgE	κ	λ	D+M+G+A+E
Patients									
SB	0·3	12·8	6·9	3·9	1·8	N.D.	10·7	9·4	25·4
AL	0·3	10	20·4	10·3	3.9	N.D.	11·4	7·2	44·6
MD	0	10·5	10·7	4·4	1·9	0·3	9·6	4·5 ©	27·8
JS	0	15·3	5	6·6	1·8	0·4	7·8	4·4	29·2
JR	0	6·3	4·9	3·9	1·5	0	5·4	1·9	16·6
JH	0	4·9	3·9	3·8	2·4	0·8	3·2	4·6	15·8
JK	0·2	16·4	14·8	7·8	2·6	0·4	6·9	5·5	42
KJ	0	13·0	9·4	12·6	2·1	0·4	5	10·8	36·1
LH	0	10·1	16	8·7	0·7	0·9	7·8	5·8	36·4
GA	0	6·7	12	10·6	1·4	0 7	4	7 6	31 4
AJ	0·3	9·5	10·7	4·4	3·2	0	11·3	3 9	27·8
MM	0·3	11·4	10·7	2·8	2·0	0	7·9	5·8	26·9
Controls									
TH		2·7	2·4	1·9	1·3		4·4	4·2	8·3
NL		4·4	4·2	3	0·5		4·9	2·8	12·1
JC		1 5	0	6·3	3·6		4·2	0·5	11·4
RJ		1 3	3·4	2·6	1·8	1	5·4	2·9	10·1
CK		5·9	3·0	0·99	3·2		5·7	1·5	9·8
ID		9·5	13·3	1	0·9	0·5	11	5·6	27·6
MS		5·2	5·8	1·9	0·5	1	13·0	3·9	14·8
LT		4·4	4·4	4·4	1·5	1·0	9·0	2	14·7
SB		6·8	11·0	3·5	2·8	0·5	6·4	4·2	23·3
LF		3·8	6·4	3·9	1	0·5	5·3	4	17·4
NW		4·3	5·9	2·4		1·4	10·0	6·1	15·0

N.D.—not determined.

and eleven normal individuals. The protocol and reagents used in the rosetting technique were standardized over the period of this study. A summary of the relevant clinical data for twelve patients diagnosed as having active contact dermatitis is presented in Table 1. Patients S.B., G.A., M.M. and possibly A.J. had evidence of endogenous eczematous dermatitis. Patients S.B., J.H., A.J. and M.M. had a positive family history of atopy, i.e. eczematous dermatitis, and/or bronchial asthma and/or allergic rhinitis. Subjects S.B. and A.J. were probably mildly atopic, whilst G.A. had venous stasis dermatitis. The blood lymphocyte data are presented in Table 2 and Fig. 1. It is apparent that there is a marked elevation in the percentage of cells bearing IgD, IgM and IgG with mean values of 10·6%, 10·4% and 6·6% respectively compared with mean values of 4·7%, 6% and 2·5% obtained for the normal subjects. These values are reflected in elevated numbers of B cells detected using anti-κ and anti-λ reagents, but it must be emphasized that the total percentage of Ig bearing cells given by the sum of values obtained with the anti-κ and anti-λ reagents is in all cases lower than the sum of the values obtained for each heavy chain class and thus the majority of cells are expressing more than one isotype. In two patients, S.B. and J.S., $\delta+$cells predominate and it would appear that in these patients there may exist a population of cells bearing IgD only. In patient A.L. most or all cells are $\mu+$ and consequently some cells must express more than two Ig classes on their membranes. It is of particular interest that patients A.L., K.J. and G.A. show a marked elevation in cells bearing IgG and also that for patients K.J. and G.A. this is associated with an inversion of the normal ratio of kappa to lambda expression. Therefore, in summary, although these patients share many clinical features and are mostly sensitized to nickel or chromate the sIg profile of lymphocyte preparations is very variable and contrasts markedly with that obtained with normal control preparations. The values obtained for

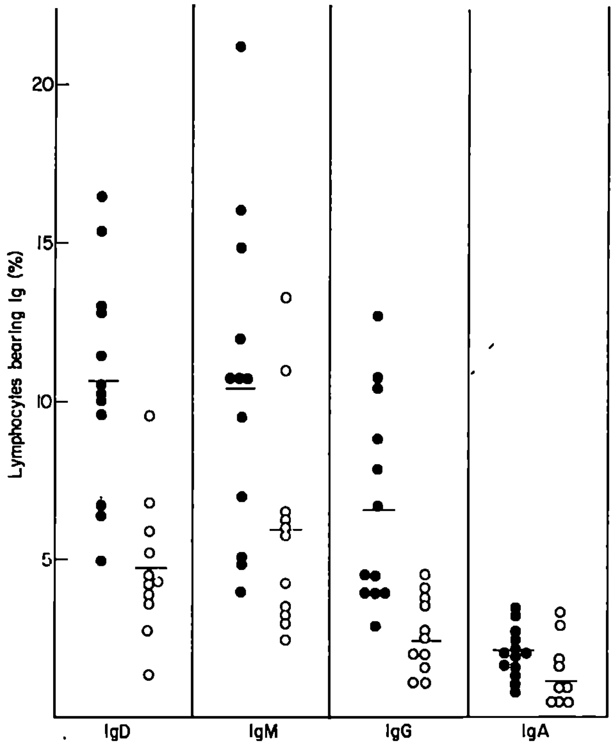

FIGURE 1. Percentage of peripheral blood lymphocytes bearing Ig isotypes. (●) Patient group; (○) control group.

TABLE 3. Serum immunoglobulin levels

Patient	IgG (g/l)	IgA (g/l)	IgM (g/l)	κ/λ ratio	IgD (μg/ml)
SB	12·1	1·46	0·82	2:1	150
MD	15·7	1·52	0·48	2·5:1	<18
JS	8·4	1·24	1·1	2:1	<18
JR	12·1		1·4	1·7:1	<18
JH	12·1	2·1	0·84	2:1	<18
JK	14·5	3·6	2·2	2:1	65
KJ	15·1	0·58	3·43	2:1	<18
LH	12·7	1·08	0·76	1·2:1	<18
GA	12·2	0	0·3	2:1	<18
AJ	9·0	2·91	0·82	1·8:1	<18
MM	9·0	1·45	1·92	1·3:1	<18

sIgE bearing lymphocytes are at background levels and support the diagnosis of contact dermatitis rather than atopic dermatitis in which the numbers of sIgE bearing cells present in peripheral blood is markedly raised (Carapeto *et al.*, 1976).

A profile of the serum immunoglobulin levels is presented in Table 3. With the exception of patient G.A. who was shown to have a selective IgA deficiency the IgG, IgM, IgA and IgD values obtained for all patients was within the normal range.

DISCUSSION

In the present study the mean values obtained for sIgD, sIgM and sIgG bearing lymphocytes in the peripheral blood of patients with active contact dermatitis were 10·6%, 10·4% and 6·6% respectively. This contrasts with values of 4·7%, 6·0% and 2·5% obtained for normal controls evaluated by an identical technique. It can be seen from Fig. 1 and Table 2 that the range of values obtained for the normal and patient groups overlap, but that the values for the patient group include some individuals with particularly elevated levels of lymphocytes bearing a given heavy chain isotype. The elevation of sIg bearing lymphocytes is likely to be a transient phenomenon related to the severity of disease and its duration etc. Therefore, although the patient group all had active disease and had not been treated they may not be expected to be a homogenous group.

The proportion of sIg bearing lymphocytes and the pattern of heavy chain isotype expression is of particular interest in relation to the previously reported studies of Cormane *et al.* (1975) and Carapeto *et al.* (1976). In the present study the total sIg positive lymphocyte population is given by the sum of the values obtained for kappa and lambda light chain bearing cells. It is well established that whilst an individual lymphocyte may express more than one heavy chain isotype it only expresses one light chain isotype (Kunkel, 1976; Preud'homme, Brouet & Seligman, 1977; Gathings, Lawton & Cooper, 1977). Cormane *et al.* (1975) and Carapeto *et al.* (1976) assumed that each lymphocyte expresses only one heavy chain isotype and consequently calculated the total sIg lymphocyte population as the sum of cells bearing individual heavy chain isotypes.

It is of particular interest to consider what population of sIg bearing lymphocytes is expanded in contact dermatitis. Cormane *et al.* (1975) and Carapeto *et al.* (1976) reported that an expanded population of cells bearing IgD only accounted for the increased sIg bearing lymphocyte count. In the study of Cormane *et al.* (1975) the percentage of cells bearing IgD is higher than the sum of cells bearing the other heavy chain isotypes and hence it would appear that a population of IgD only bearing cells are being detected. It should be noted that these authors do report very high values for IgD bearing cells (mean 38%, range 19·5–87%). In the study of Carapeto *et al.* (1976) the percentage of cells bearing IgD is less (mean 13·5%) than the sum of the other heavy chain isotypes detected (mean 17·6%) and thus it cannot be concluded that an IgD only bearing population was detected. In the present study comparison of the values obtained for the individual heavy chain isotypes with that for the total sIg population given by the kappa plus lambda values was made to evaluate the pattern of sIg expression. Only patients S.B. and J.S. have values for sIg bearing cells that are notably higher than any other individual heavy chain isotype and thus may have a population of sIgD only bearing cells. Whilst patient J.K. has a high IgD bearing population of cells (16·4%) this is about the same value obtained for the total sIg bearing population and so all IgD bearing cells must also express at least one other heavy chain isotype. This analysis contrasts sharply with that obtained if the total sIg lymphocyte population is assumed to be the sum of the values obtained for the individual heavy chain isotypes.

The high values of sIgG bearing lymphocyte detected in patients A.L., K.J. and G.A. is of interest. Since it was recognized that there exists a population of lymphocytes that can bind IgG non-specifically through an Fc receptor (Lobo *et al.*, 1975; Winchester *et al.*, 1975) the technique employed in the preparation of lymphocytes from peripheral blood has been adapted so that such cytophilically bound IgG is removed from the cells. Using present standard methodology and immunofluorescence for the detection of sIg, several groups report values for sIgG bearing lymphocytes <1·0% (Kunkel, 1976; Preud'homme *et al.*, 1977). However, using the sensitive rosette technique Dhaliwal *et al.* (1978) did detect a significant population of IgG bearing cells in normal peripheral blood lymphocytes and also on a high proportion of cells from some patients with chronic lymphocytic leukaemia. The rosette technique employed in the present study gave a range of values for sIgG on normal lymphocytes of 1–4·4%. The values of 10·3%, 12·6% and 10·6% obtained for patients A.L., K.J. and G.A. represent a marked increase in sIgG bearing cells above normal. The values obtained for sIgA and sIgE bearing cells were all within the normal range. A further feature worthy of comment is that for three individuals within this small patient group (J.A., J.H. and G.A.) cells bearing lambda light chains predominated over kappa bearing chains; a reversal of the normal situation.

In contrast to the variable pattern of sIg expression on peripheral blood lymphocytes the corresponding serum immunoglobulin levels show less variation. Most patients had IgG, IgM and IgA levels within the normal range whilst one individual, G.A., had a selective IgA deficiency.

The occurrence of a predominant sIgD only positive lymphocyte population as a feature of patients with contact dermatitis has not been clearly established in the present study and it has been argued also that the data are consistent with a re-interpretation of the study of Carapeto *et al.* (1976). In the active stage of the disease it may be that a non-specific increase in sIg positive lymphocytes in peripheral blood may be observed but that it is not of significance to any underlying immunological mechanism. Further studies should be directed towards detecting the presence and activity of lymphocytes in the lesion since it has been claimed (Cormane *et al.*, 1975) that sIgD bearing cells predominate in these situations.

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Effect of five anti-inflammatory steroids on collagen and glycoaminoglycan synthessis *in vitro*

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SUMMARY

The effect of five anti-inflammatory corticosteroids, i.e. hydrocortisone, hydrocortisone 17-butyrate, betamethasone 17-valerate, nicocortonide acetate and nicocortonide, on the synthesis of hyaluronic acid, sulphated glycosaminoglycans and collagen by cultured skin fibroblasts was studied. As inhibitors of all these parameters the steroids could be arranged in order hydrocortisone < hydrocortisone 17-butyrate < betamethasone 17-valerate, nicocortonide acetate and nicocortonide. The corticosteroid concentrations required for inhibition of hyaluronic acid were very low as compared to those required for inhibition of sulphated glycosaminoglycan and collagen synthesis.

New derivatives of hydrocortisone with higher anti-inflammatory potency are constantly being developed. In order to guide the clinician in the selection of steroids for therapy, the anti-inflammatory potencies of the steroid molecules *per se* as well as of their topical formulations need to be evaluated. Similarly, efforts must be made to assess those steroid effects which are usually considered to be unwanted side-effects, e.g. skin thinning, telangiectasia, etc. These are due to inhibition of epidermal cell proliferation, to a decrease in the number and the dividing rate of dermal fibroblasts, as well as to decreased synthesis of the extracellular matrix components, e.g. collagen and glycosaminoglycans (Nacht & Garzon, 1974). In the present study we have compared the ability of five anti-inflammatory steroids to affect connective tissue by measuring their effects on collagen and glycosaminoglycan synthesis in human skin fibroblast cultures.

MATERIAL AND METHODS

Cells

Fetal human skin fibroblasts, obtained from a normal 5-month-old human fetus, at 6–10 passages were cultured in glass bottles containing Dulbecco's modification of Eagle's minimal essential medium supplemented with 10% fetal calf serum (both medium and serum were from Flow Laboratories, Irvine, United Kingdom) buffered with 23 mM sodium bicarbonate and 20 mM HEPES to pH 7·3.

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Penicillin (100 μg/ml) and streptomycin sulphate (50 μg/ml) were added to the medium. The same cell strain was used throughout the study.

Collagen synthesis

The cells were detached with 0·25% trypsin, suspended in the medium and pipetted in 200 μl aliquots into the wells of cell culture plates (Falcon Microtest II, B.-D., U.K. Ltd, Wembley, United Kingdom) with a growth area of 0·3 cm² per well. Five days later dense confluent monolayers were found. The medium was then replaced with 200 μl of fresh serum containing medium with 50 μg/ml freshly solubilized ascorbic acid, 0·5 μCi/ml ^{14}C-proline (L-U-^{14}C proline spec. act. 274 mCi/mmol, The Radiochemical Centre, Amersham, United Kingdom) and the test compounds. The steroid concentrations in the final growth medium were 1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M and 1×10^{-4} M. No steroid was added to the controls. The plates were then incubated for 24 h at 37°C. After incubation the medium from each well was collected and the amount of radioactive collagen in them was determined by the method of Jalkanen *et al.* (1980). The pepsin resistant (digested at 4°C for 3 h) helical sequences of secreted collagen molecules were precipitated with 0·6 M perchloric acid onto glass fibre discs. The free radioactivity was washed away and the radioactivity left on the membrane was counted in a scintillation spectrometer.

Glycosaminoglycan synthesis

After trypsinization the cells were seeded into plastic cell culture tubes with 5·5 cm² growth area and were grown until confluent. The medium was then changed to 2 ml of fresh serum-containing medium containing the steroids and the isotope (1·5 μCi/ml, ^{3}H-glucosamine hydrochloride, spec. act. 3·2 μCi/mmol, The Radiochemical Centre, Amersham, United Kingdom). The final steroid concentrations in the medium were 1×10^{-9} M, 1×10^{-8} M, 1×10^{-7} M, 1×10^{-6} M and 1×10^{-5} M. The tubes were incubated for 24 h at 37°C. At the end of the incubations the media were collected. Four hundred microlitres of them were papain-digested (500 μl/sample, 60,000 u/g, Merck, Darmstadt, Germany) for 6 h at 60°C in the presence of 0·005 M Na-EDTA and 0·005 M cysteine-HCl. After cooling the samples to room temperature the glycosaminoglycans were precipitated with cetylpyridinium chloride (Merck) and fractionated to hyaluronic acid and sulphated glycosaminoglycans by the method of Saarni & Tammi (1977).

Steroids

The steroids studied were hydrocortisone (Merck), hydrocortisone 17-butyrate (Gist-Brocades, Delft, Holland), betamethasone 17-valerate (Glaxo, Greenford, Middlesex, United Kingdom), and the 'ansa' corticosteroids 14α, 17α-(2-butenylidenedioxy)-11β-hydroxy-3,20-dioxopregn-4en-21yl-isonicotinate for which the WHO proposed name is 'nicocortonide', and the corresponding 21-acetate (as yet unnamed Gist-Brocades). All the steroids were dissolved in DMA (N, N,-dimethylacetamide, Koch-Light Laboratories, Colnbrook, Bucks, United Kingdom) so that the final media contained 5 μl/10 ml in every case. The control media contained only this DMA-concentration without any steroid.

RESULTS

Collagen synthesis

The synthesis of collagen was not significantly affected by hydrocortisone or hydrocortisone 17-butyrate at any of the tested concentrations (Table 1 and Fig. 1). Betamethasone 17-valerate, nicocortonide and its 21-acetate, on the other hand, strongly inhibited the synthesis of collagen at a

TABLE 1. Effects of anti-inflammatory steroids on collagen synthesis. The values are expressed as c.p.m. ± s.d. n = 16 in controls and 6 in steroid experiments. The control value was 1359·1 ± 129·0

Steroid	Steroid concentration (M)			
	1×10^{-7}	1×10^{-6}	1×10^{-5}	1×10^{-4}
Hydrocortisone	1492·2 ± 171·3	1508·1 ± 179·1	1492·9 ± 116·4	1395·5 ± 130·1
Hydrocortisone 17-butyrate	1554·1 ± 85·7	1441·1 ± 208·8	1356·4 ± 167·2	1469·1 ± 126·6
Betamethasone 17-valerate	1411·8 ± 120·0	1372·5 ± 188·4	1443·7 ± 194·1	473·1 ± 89·5*
Nicocortonide acetate	1408·7 ± 142·6	1367·7 ± 185·0	1349·1 ± 151·7	672·0 ± 237*
Nicocortonide	1442·6 ± 111·7	1503·1 ± 82·5	1328·3 ± 114·6	427·2 ± 108·9*

* $P < 0·05$ (Student's '*t*'-test).

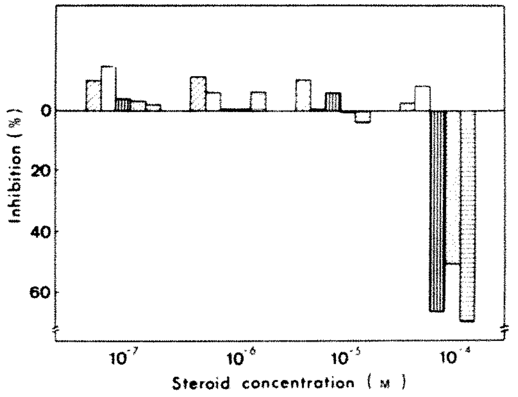

FIGURE 1. The relative inhibitory action of the five steroids in the synthesis of collagen expressed as per cent of control. (Hatched columns) hydrocortisone; (open columns) hydrocortisone 17-butyrate; (striped columns) betamethasone 17-valerate; (stippled columns) nicocortonide acetate; (horizontal striped columns) nicocortonide.

concentration of 10^{-4} M (the inhibition percentages were 66, 69 and 51, respectively, when compared to the controls). Lower concentrations of these steroids were non-inhibitory in this test system. There were thus no marked differences between these three latter steroids with regard to their ability to inhibit collagen synthesis. Marked inhibition could however be recorded with all of the steroids if higher concentrations were used.

Glycosaminoglycan synthesis

At concentration 10^{-9} M, hydrocortisone and hydrocortisone 17-butyrate did not inhibit the synthesis of hyaluronic acid (Table 2 and Fig. 2). The other tested steroids, betamethasone 17-valerate, nicocortonide and its 21-acetate, however, inhibited it by 16%, 23% and 26%, respectively. At the concentration 10^{-8} M all the steroids, except hydrocortisone, were clearly inhibitory (the inhibition was 30–50%). At higher concentrations (10^{-7} M to 10^{-5} M) all the tested steroids inhibited the synthesis of hyaluronic acid by about 30–40%. No definite differences could be recorded between the steroids at this concentration range. Higher concentrations than these would be needed for a more marked inhibition.

The synthesis of sulphated glycosaminoglycans was inhibited only at the highest concentration,

TABLE 2. Effects of anti-inflammatory steroids on the synthesis of hyaluronic acid. The values are expressed as c.p.m. ± s.d. n = 9 in controls and 4 in steroid experiments. The control value was 1199·2 ± 202·9

Steroid	Steroid concentration (M)				
	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Hydrocortisone	1257·3 ± 161·2	1258·1 ± 123·7	788·5 ± 67·4*	910·6 ± 98·7*	675·5 ± 75·0*
Hydrocortisone 17-butyrate	1282·3 ± 114·9	790·6 ± 86·9*	834·5 ± 95·4*	797·1 ± 90·8*	695·5 ± 107·2*
Betamethasone 17-valerate	1002·2 ± 145·3	832·5 ± 51·6*	756·1 ± 123·2*	746·5 ± 54·7*	610·8 ± 24·7*
Nicocortonide acetate	888·6 ± 141·3*	760·2 ± 68·4*	836·5 ± 41·6*	818·7 ± 94·5*	663·9 ± 62·2*
Nicocortonide	926·5 ± 22·3*	568·2 ± 76·5*	686·1 ± 198·7*	671·3 ± 259·6*	652·6 ± 33·5*

* $P < 0·05$ (Student's '*t*'-test).

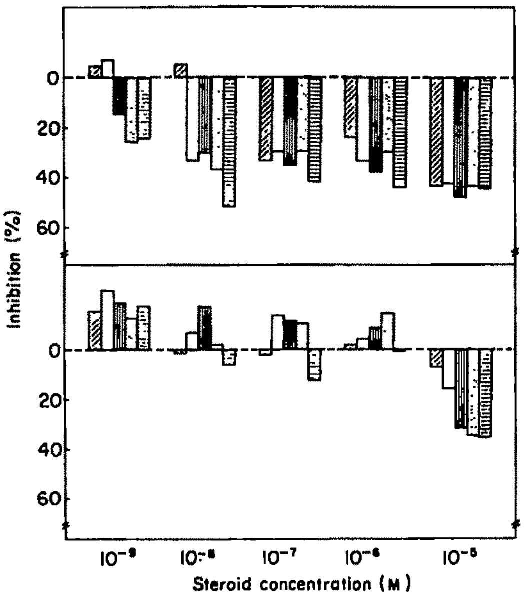

FIGURE 2. The relative inhibitory action of the five steroids on the synthesis of hyaluronate (above) and sulphated glycosaminoglycans below) expressed as per cent of control. (Hatched columns) hydrocortisone; (open columns) hydrocortisone 17-butyrate; (striped columns) betamethasone 17-valerate; (stippled columns) nicocortonide acetate; (horizontal striped columns) nicocortonide.

i.e. 10^{-5} M (Table 3 and Fig. 2). Hydrocortisone and hydrocortisone 17-butyrate were slightly less inhibitory than the other three steroids, which inhibited the synthesis of sulphated glycosaminoglycans by about 35%.

DISCUSSION

In the present study the incubation time was kept at 24 h to minimize the well-known steroid effect on cell division and cell numbers (Ponec *et al.*, 1977; Saarni, 1977; Kruse *et al.*, 1978; Priestley,

TABLE 3. Effects of anti-inflammatory steroids on the synthesis of sulphated glycosaminoglycans by cultured fibroblasts. The values are expressed as c.p.m. ± s.d. n = 9 in the controls and 4 in steroid experiments. The control value was 301·87 ± 52·3

Steroid	Steroid concentration (M) 10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Hydrocortisone	349·9 ± 42·0	298·1 ± 40·4	296·0 ± 36·9	308·3 ± 18·0	281·7 ± 48.7
Hydrocortisone 17-butyrate	390·8 ± 49·2	323·3 ± 39·4	345·5 ± 15·2	316·2 ± 45·2	255·9 ± 38·1
Betamethasone 17-valerate	360·5 ± 53·7	354·3 ± 36·8	339·4 ± 40·8	329·6 ± 32·6	208·0 ± 40·3*
Nicocortonide acetate	344·0 ± 34·5	308·5 ± 39·7	337·1 ± 21·2	349·9 ± 23·8	198·9 ± 29·7*
Nicocortonide	356·8 ± 30·7	286·1 ± 20·0	266·6 ± 70·8	301·7 ± 55·1	196·1 ± 13·8*

* $P < 0{\cdot}05$ (Student's '*t*'-test).

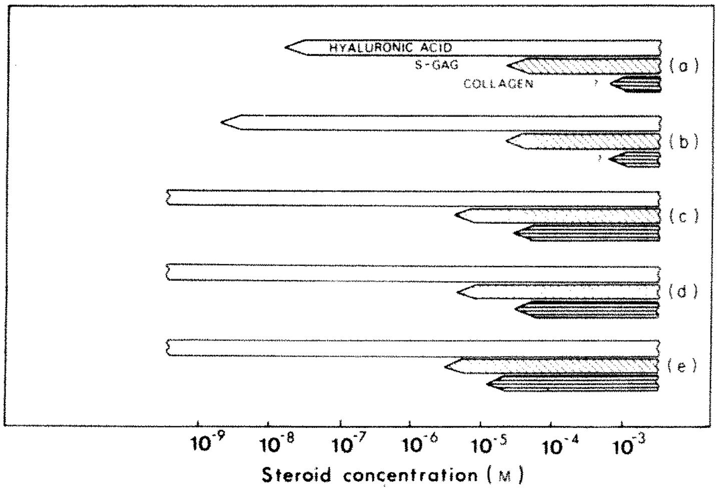

FIGURE 3. The concentrations of the tested steroids needed to cause a significant inhibition in the collagen, hyaluronic acid and s-GAG synthesis. (a) Hydrocortisone; (b) hydrocortisone 17-butyrate; (c) betamethasone 17-valerate; (d) nicocortonide acetate; (e) nicocortonide.

1978). That is also one of the reasons why confluent cultures were used. All the determinations were carried out from the culture medium, because we have earlier shown that changes in the amounts of medium collagen and glycosaminoglycans reflect the changes in the total synthesis of these substances (Saarni, 1977; Saarni & Hopsu-Havu, 1978).

An inhibition of collagen synthesis was obtained with betamethasone 17-valerate, nicocortonide and its 21-acetate only at the highest studied concentration (1×10^{-4} M). Lower concentrations were uniformly non-inhibitory. In the case of hydrocortisone and hydrocortisone 17-butyrate even the highest tested concentrations were too low to cause inhibition of collagen synthesis in this test system. Similarly high concentrations are also needed to inhibit collagen synthesis by chick embryo tendon cells *in vitro* (Saarni, 1977). Since such high steroid concentrations are required, it seems possible that the inhibitory action of anti-inflammatory steroids on collagen synthesis is due to a general inhibition of protein synthesis by the cells without any specificity to collagen (Saarni, 1977; Kruse *et al.*, 1978), even though in some studies selectivity of collagen synthesis to the inhibitory effects of anti-inflammatory steroids has been shown (Cutroneo, Stassen & Cardinale, 1975).

The synthesis of hyaluronic acid responded to hydrocortisone at 10^{-7} M concentration and to hydrocortisone 17-butyrate at 10^{-8} M concentration. Betamethasone 17-valerate, nicocortonide and its 21-acetate were inhibitory even at 10^{-9} M concentration. The inhibition did not rise over 50% with any of the steroids at any concentrations studied. A more marked inhibition of acid mucopolysaccharides would certainly be recorded if higher steroid concentrations were used. Almost complete inhibition can be recorded at steroid concentrations which inhibit also collagen and non-collagen protein synthesis (Ebert & Prockop, 1967). An identical non-linearity in the steroid-caused inhibition of hyaluronate synthesis has been reported earlier (Castor & Dorstewitz, 1966; Saarni, 1978; Saarni & Hopsu-Havu, 1978).

The synthesis of sulphated glycosaminoglycans was inhibited only at the highest steroid concentration tested (10^{-5} M). At this concentration hydrocortisone and hydrocortisone 17-butyrate had little inhibitory effect (7 and 15%, respectively). However, the three more potent steroids, betamethasone 17-valerate, nicocortonide and its 21-acetate, inhibited much more effectively, i.e. 30–35%. Again there were no great differences between these three potent steroids.

When comparing all the results together, the synthesis of hyaluronic acid is found to be much more sensitive than the synthesis of collagen and sulphated glycosaminoglycan to the inhibitory action of steroids (Fig. 3). Hyaluronic acid synthesis was inhibited by concentrations about 10^4 times lower than those needed for inhibition of the synthesis of sulphated glycosaminoglycans and 10^5 times lower than those needed for inhibition of collagen synthesis. The discriminating concentration range in the hyaluronate effects was also wider than in the other systems studied. It differentiated well between hydrocortisone and hydrocortisone 17-butyrate on the one hand, and the three more potent steroids on the other hand. Concentrations lower than 10^{-9} would be needed in order to record differences in the activities of these latter steroids (betamethasone 17-valerate, nicocortonide and its 21-acetate). In studies of inhibitory steroid effects on collagen or sulphated glycosaminoglycan synthesis, one has to work with high steroid concentration ranges, and the recordable differences between the steroids are smaller. On the basis of the present findings it is possible to divide the tested steroids into three groups, i.e. hydrocortisone, its 17-butyrate, and the more potent steroids, but we observed no clear cut differences between the individual steroids in the latter group.

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ATP induced polarization of keratohyalin granules

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SUMMARY

Within seconds of exposure of frozen sections of stratum granulosum of glycerinized cow hoof to a buffered solution of adenosinetriphosphate, the keratohyalin granules underwent transformation into polarized fine linear rods. This polarization phenomenon was associated with gross pleating, twisting and crumpling of the section, indicative of varying contractile forces.

Although the stratum granulosum has been characterized for over a hundred years by the presence of unique basophilic granules, the nature and function of these keratohyalin granules has eluded precise definition (Ugel, 1977). Recently, we demonstrated that glycerinized stratum granulosum acts as a grossly contractile system in the presence of adenosine triphosphate (ATP) (Shelley & Juhlin, 1980). The present report details the distinctive microscopic changes observed in the granulosum cell and its keratohyalin granules at the time of contraction.

MATERIALS AND METHODS

Narrow strips of the hairless skin just behind the hard hoof of healthy young steers were cut out within two hours of slaughter. These were placed in pre-chilled 50% glycerine at 0°C for 24 h and then stored for up to 6 months at −22°C. Transverse frozen sections (7 μm) were cut through the stratum granulosum, placed in test solutions at room temperature for one minute, transferred to 25% glycerine in distilled water on a slide, straightened, fixed in 95% ethanol, stained with haematoxylin and mounted in Kaiser glycerine jelly for observation. Phase microscopy was also done on selected specimens immediately upon transfer to the 25% glycerine. In others, ATPase stains were made (Juhlin & Shelley, 1977).

The above techniques and chemicals used are detailed in the prior report (Shelley & Juhlin, 1980) on the gross findings. The contraction solution used had the following standard composition:

Mg ATP	2 mM
Calcium chloride	1 mM
Cysteine	1 mM
Na Cl	170 mM
MOPS buffer	10 mM
pH	7·2

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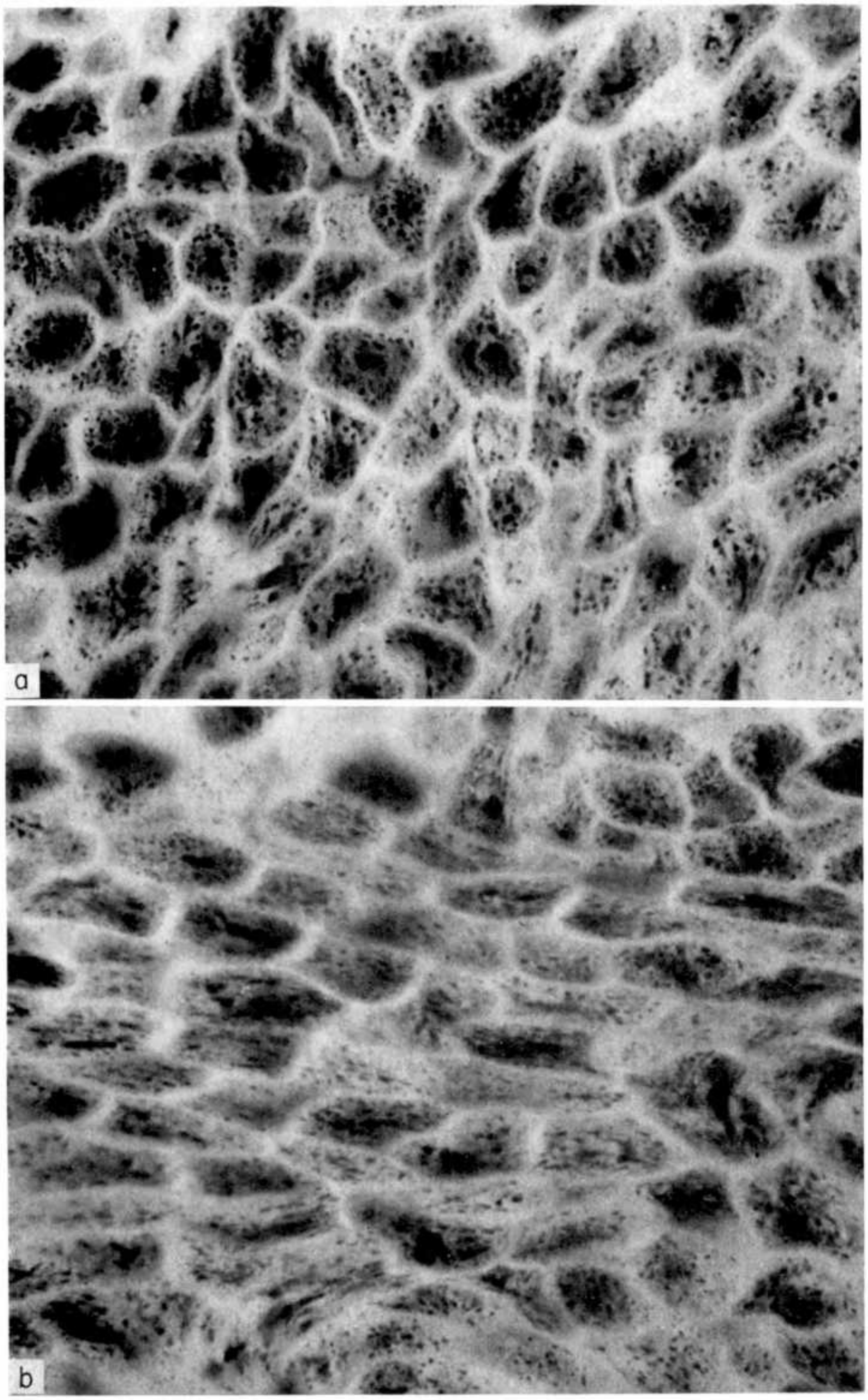

FIGURE 1. (a) Stratum granulosum in control buffer (1 mM $CaCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2). Keratohyalin granules are variably sized, irregular, and in random distribution. Cells are polygonal and round. Bovine hoof 7 μm glycerinized, frozen section, alcohol fixed (haematoxylin stain, × 530). (b) Stratum granulosum in Mg ATP (2 mM Mg ATP, 1 mM $CaCl_2$, 1 mM cysteine, 10 mM MOPS, 170 mM NaCl, pH 7·2). Keratohyalin granules are linear, and oriented with polarization in direction of long axis of keratohyaline and the elongated cells. This serial section immediately adjacent to that of Fig. 1a (Haematoxylin stain, × 530).

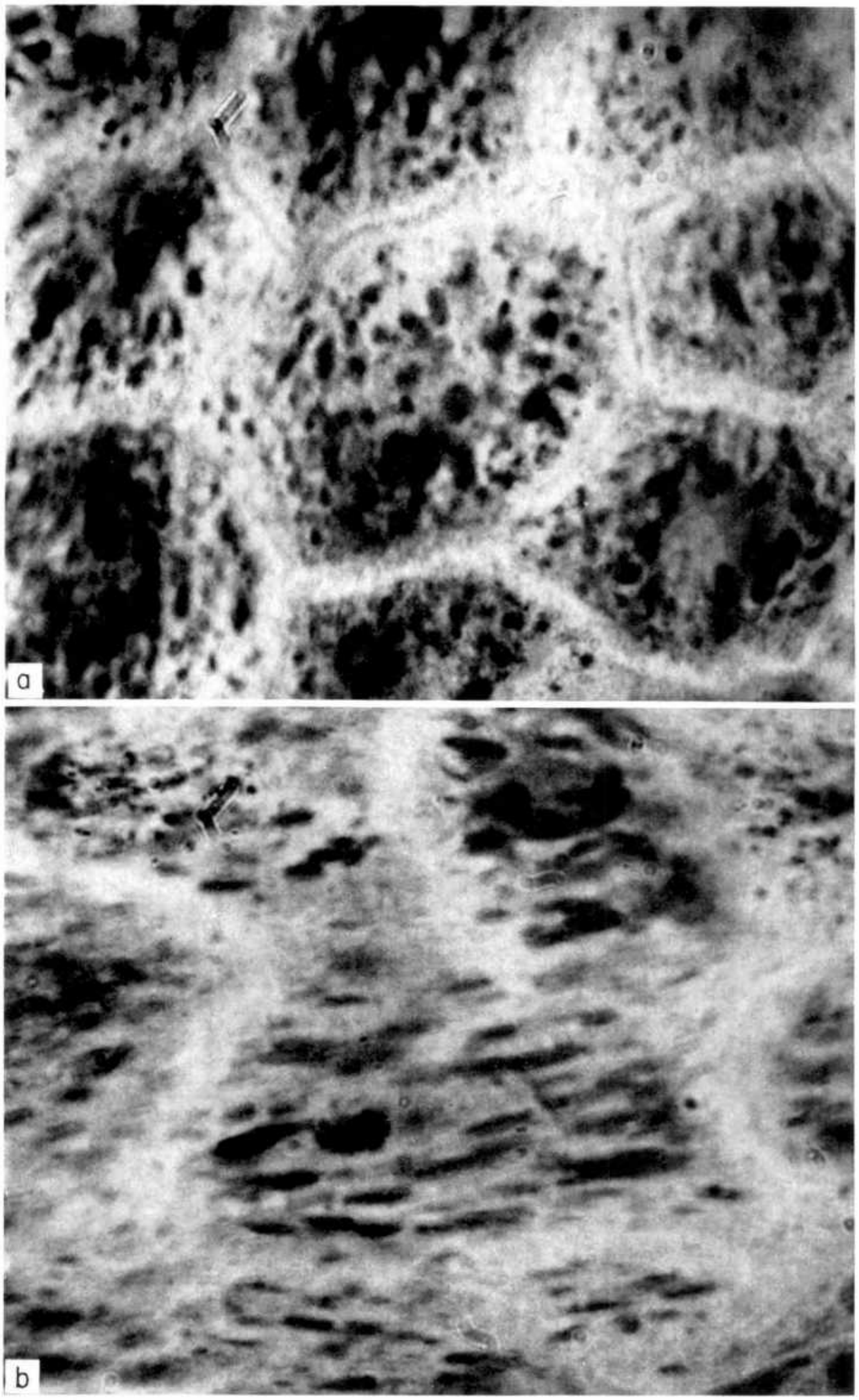

FIGURE 2. High power view of stratum granulosum cells. (a) In control buffer (10 mM HEPES in place of MOPS). Polygonal cells are seen with variably sized, irregular, randomly distributed keratohyalin. Haematoxylin stain. Serial section from block used in Fig. 1 (× 1330). (b) In Mg ATP (HEPES buffer). Keratohyalin has become linear and polarized. Haematoxylin stain. This section immediately adjacent to that in Fig. 2a (× 1330).

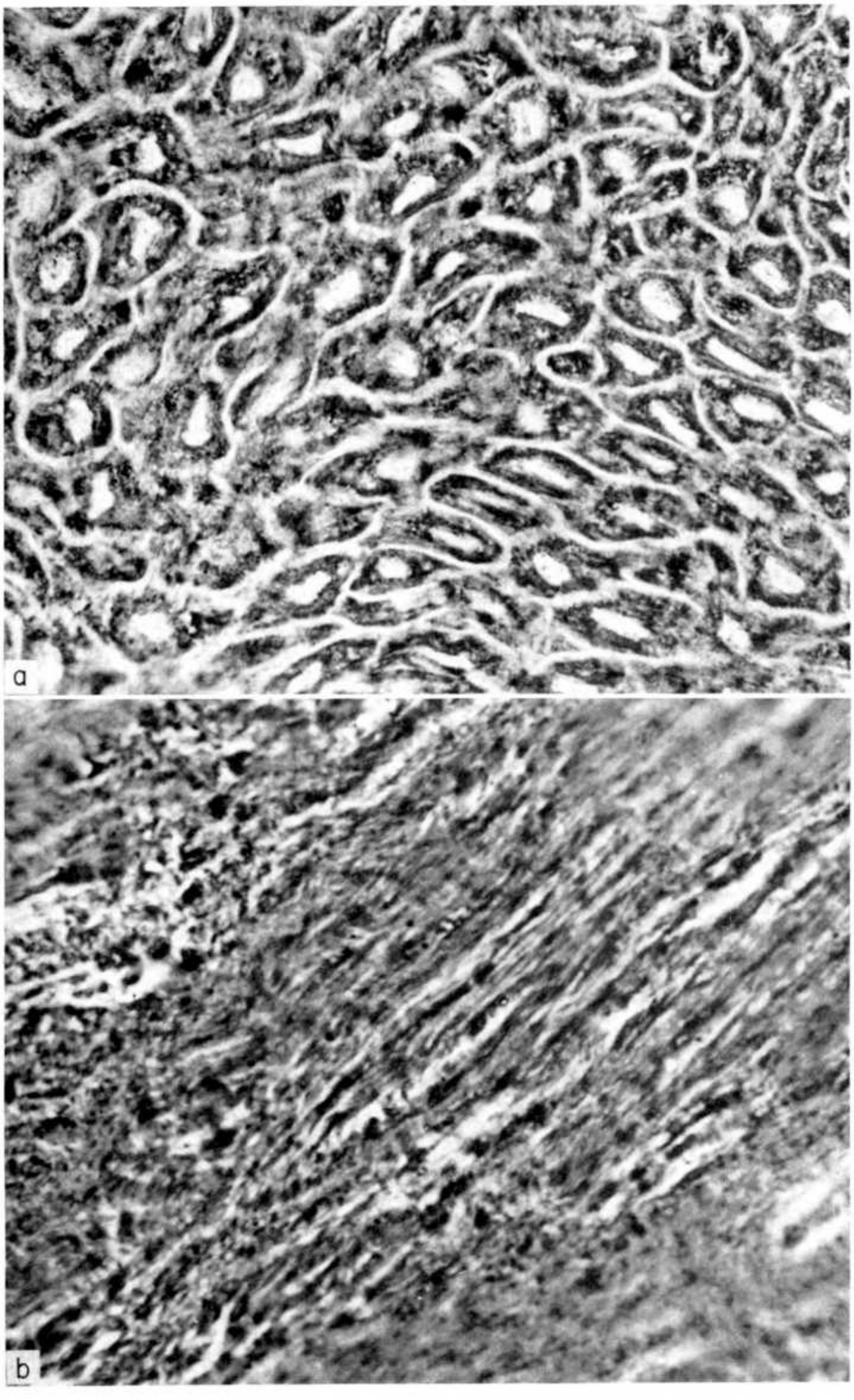

FIGURE 3. Phase-contrast view of stratum granulosum. (a) In control buffer. cells are of variegate shape. Unstained, 3 μm section (× 530). (b) In Mg ATP, cells are linear, showing streaming of thread-like keratohyalin. Note areas out of focus result from contraction during observation. Unstained, adjacent 3 μm section (× 530).

RESULTS

General observations

The contraction solution induced a narrowing of many of the normally polygonal cells of the stratum granulosum (Figs 1 and 2). Concurrently, there was a change in the shape and orientation of the keratohyalin granules. The granules lost their rounded, globoid, darkly stained conformation, becoming thin linear strips, threads, and dots. All of these became distinctly polarized in an axis parallel to the long axis of the cell. These transformed rod-like granules seem to be streaming, being lined up in a fashion similar to iron filings in a magnetic field. At times this gave the appearance of combed hair or a 'stratum striatum'. Groups of cells showed similar internal alignments, but such bands of cells with identical polarization could be seen intersecting with others of differing alignment. The cell nuclei or nuclear spaces were often similarly elongated and oriented in the same pathway as the keratohyalin granules.

These changes were not seen in stratum spinosum and were less evident in the lowermost levels of cells of the stratum granulosum where the keratohyalin granules were but fine dots. The changes did not occur regularly in solutions in which Mg ATP was absent. The degree of polarization invariably correlated with the degree of contractility exhibited by the specimen.

Phase microscopy on 3 μm sections confirmed these findings (Fig. 3).

Specimen

The histological change was more evident and striking in sections which were cut parallel or oblique to the skin surface. In areas of diseased skin exhibiting parakeratosis the histological and gross responses were both absent. Specimens which had thawed or had been pretreated with formalin did not exhibit the phenomenon.

Observations were made on sections 3, 5, 7, 8, 10, 14 and 20 μm thick. The 3 and 5 μm sections were hard to handle, becoming twisted and contracted into a wad which could not readily be flattened for staining. Sections at 7, 8, 10 and 14 μm were good, but the 20 μm sections proved too thick for ideal staining.

Time

The reaction was rapid occurring in less than 30 s. Specimens kept in the solution for 5 min showed less gross waffling on the slide and the granules became finer, smaller rods. After 10 min the tissues were flat and more rounded granules were seen.

Temperature

The polarization of granules was demonstrable at room temperature, 22° and at 37°C. It was not seen in contraction solution kept at 0°C.

ATP

The polarization response could be perceived at concentrations of Mg ATP as low as 0·5 mM. It appeared maximal at 2 mM. Concentrations of 5 mM produced so much contraction of the sections as to make staining unsatisfactory. Substitution of ADP for the ATP eliminated the histological response. However, INT could replace ATP in its ability to elicit the polarization phenomenon.

Buffers

The phenomenon was best seen using MOPS or PIPES as a buffer. It was also clearly present with Tris maleate, HEPES and Veronal (Michaelis) buffers. The Sorenson phosphate buffer seemed the least satisfactory.

pH

Using Veronal (Michaelis) buffer, polarization was studied in a 2 mM Mg ATP, 1 mM calcium, 1 mM cysteine system at pH 4·0, 5·0, 6·0 and 7·0. The response was seen at all of the pH levels, being maximal at pH 6·0.

Ions

Removal of the calcium ion greatly reduced the polarization response, whereas removal of the cysteine had a lesser but still observable effect in reducing the response to Mg ATP. Substitution of K (50 mM) for the Na (170 mM) eliminated polarization.

DISCUSSION

The present report describes a new and unexpected phenomenon, viz. the elongation and parallel alignment of keratohyalin granules. This intracellular conformational change of striking geometric precision is induced by ATP. It appears related to the gross movement that a sheet of stratum granulosum cells exhibits upon exposure to ATP.

A key requisite for the demonstration of this polarization of keratohyalin granules and the concomitant contraction of the granulosum cells has been our adaptation of the glycerination technique long used in studies on the nature of muscle contraction (Bendall, 1969). In this procedure glycerinized muscle cell wall becomes permeable to ATP and essential cations, while the fibres retain their actin and myosin in functional array. The glycerol further acts as a neutral preservative by inactivating the mitochondria and removing most of the soluble glycolytic enzymes. Accordingly, the treated fibres acquire a responsivity to ATP.

We adapted this procedure to skin, by using frozen sections to isolate the various layers of epidermis (Shelley & Juhlin, 1980). We found the stratum granulosum to undergo a variety of gross changes ranging from scalloped borders and trough formation, to pleating, twisting, crinkling, furling and even wadding up into a small ball. This we have labelled contraction although it is evident that it is not a simple shortening of the long narrow single muscle fibre as seen by the muscle physiologist. Rather, it is the conformational change undergone by a sheet containing hundreds of cells capable of generating force vectors in a variety of directions.

It is easy to assume that a contractile protein is involved in this sheet motion since all eukaryotic cells are now known to contain this (Adelstein, Scordilis & Trotter, 1979; Pollard, 1975). Yet the response is unique, occurring only in the stratum granulosum. These granulosum cells must be highly specialized in the direction of the transduction of chemical energy into mechanical forces. They do contain ATPase (Fig. 4). One immediately asks whether the specialized cell bodies, the keratohyalin, of this layer play a role in this phenomenon. The present studies show that the granules do change both in shape and alignment during this contractile process. This could reflect a passive change of the oil-like granules in response to changes in the cell shape from polygonal to fusiform. However, the apparent loss of keratohyalin material suggests that the granule may be an active participant.

In such a highly complex system many factors are operative. These include the ionic composition, pH, buffers, and temperature of the bathing solution. Thus, the magnesium–calcium activates the ATPase and the cysteine antagonizes -SH inhibition. Although muscle physiologists routinely wash the glycerine out of the single fibre before testing, we have been unable to do this with our sheets of stratum granulosum, possibly because the cell walls are sectioned.

The phase microscopy made it evident that the phenomenon with which we have been working is not an artifact of fixation or staining, but actually is observable in live unstained stratum granulosum. Furthermore, the response was not species limited, but occurred in other animals including man. In

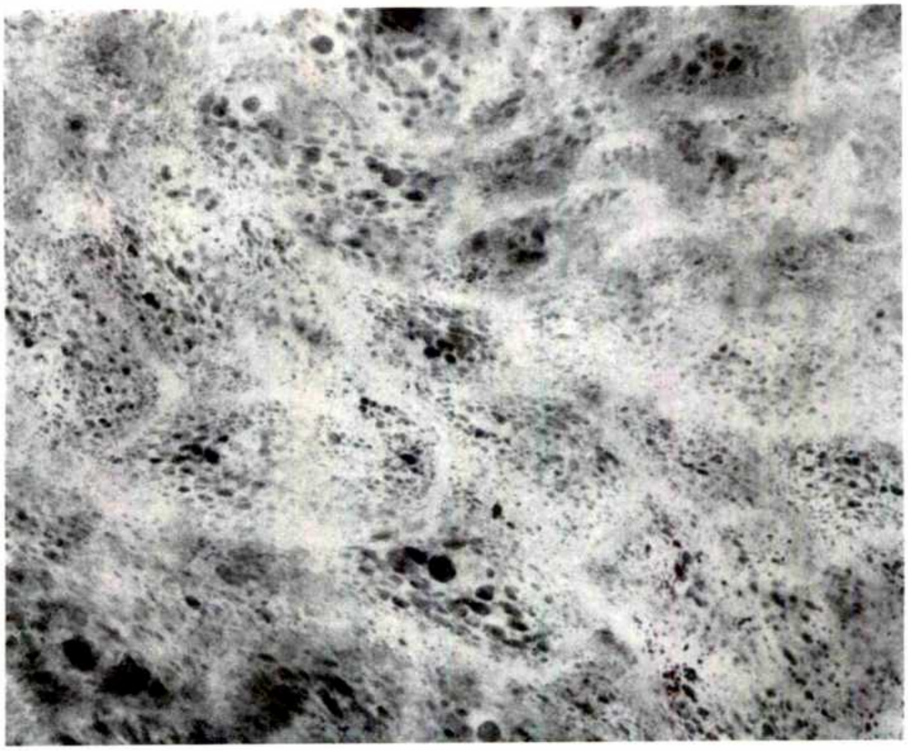

FIGURE 4. ATPase stain of stratum granulosum. Cell granules show stain for magnesium activated ATPase. Non-pigmented bovine hoof, 14 μm, 240 min incubation (× 530).

the latter instance, the studies were done most easily on the scrotum in which the stratum granulosum is about eight cells thick, in contrast to the three or four cell thickness of the layer in most human skin (Ferriera-Marques & Parra, 1960).

Significantly, an analogue of keratohyalin, trichohyalin, found in the hair sheath, may present linear rod-like granules oriented with the long axis of its cell (Rogers & Harding, 1976).

Functional analogues of the stratum granulosum cell itself are the myoepithelium surrounding the ectodermal glands (Hurley & Shelley, 1954), the myofibroblast of wound healing (Gabbiani, 1979), and the interstitial cell of the lung (Kapanci *et al.*, 1979). All of these have the specialized capacity to contract. However, in these examples the cell shortens. In the case of the granulosum cell the contraction appears to result in a flattening of the cell rather than shortening.

It is this flattening that gives clue to at least one function for the ATP response observed. We suspect that the stratum granulosum cell flattens as it passes into the dead stratum corneum, thus providing an enlarged plate shield that would otherwise be absent were the cell simply to die and shrivel passively. Contractility of these outermost live cells would also contribute to keeping the epidermis 'skin tight' during the physiological stretching and shrinkage which occur in skin. A contractile stratum granulosum, furthermore, could be a determinant of the size of the epidermal intercellular spaces. In agreement with this is the fact that psoriasis, a disease in which the stratum granulosum is absent, exhibits large intercellular spaces.

It appears that the ATP regulated transformation of randomized variably sized keratohyalin granules into highly oriented linear rods deserves further study and especially at the ultrastructural level.

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The prevalence of splinter haemorrhages

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SUMMARY

A study was undertaken to determine the prevalence and distribution of subungual splinter haemorrhages in healthy individuals. They were found commonly in both sexes, though significantly more frequently in males. Where present in females, they were confined to a single digit.

The pathogenesis of splinter haemorrhages remains an enigma. Originally described by Sir Thomas Horder in his classic description of infective endocarditis (Horder, 1920) they came to be regarded as one of the hallmarks of this condition. Subsequently they have been reported in a wide spectrum of apparently unrelated disorders (Kilpatrick, Greenberg & Sanford, 1965; Platts & Greaves, 1958) and in healthy individuals (Platts & Greaves, 1958; Robertson & Braune, 1974). Their value as a clinical sign of systemic disease has been limited by lack of knowledge of their prevalence and distribution in normal subjects.

SUBJECTS AND METHODS

The finger-nails of 121 healthy subjects were inspected for the presence of subungual splinter haemorrhages. Following the practice of Dowling (Dowling, 1964) all splinter haemorrhages visible with the naked eye in good illumination were included, irrespective of their location in the nail, or their colour. Particular care was taken to exclude ingrained dirt under the nail, where necessary by gently cleaning the nails. Toe-nails were not inspected (Gross & Tall, 1963). Seventy-one of the subjects were male (age range 18–60 years; mean 30·6 years) and fifty female (age range 19–61 years; mean 28·0 years). Twenty-three of the subjects were doctors, nineteen nurses, and eleven medical students. The remaining sixty-eight were blood donors whose occupations were not systematically recorded.

RESULTS

Splinter haemorrhages were found in twenty-five males (35%) and six females (12%), a statistically significant difference between the sexes ($P = <0{\cdot}05$) (see Table 1). Those with and without splinter haemorrhages did not significantly differ in age. In eighteen of the twenty-five males with splinter

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TABLE I.

	No.	%	Age range (years)	Mean age (years)
Males				
Total subjects	71	100	18–60	30·6
Splinter haemorrhages present	25	35	20–60	34.7
Splinter haemorrhages on more than one digit	7	10	22–55	39·5
Females				
Total subjects	50	100	19–61	28.0
Splinter haemorrhages present	6	12	21–48	28.6
Splinter haemorrhages on more than one digit	0	0	—	—

haemorrhages (72%), and in all six females, the lesions were confined to a single digit. Only four subjects (all males) had lesions on both hands. Unilateral splinter haemorrhages did not occur significantly more commonly on the dominant hand, seventeen being on the dominant hand and ten on the non-dominant. No individual digit was excessively frequently or infrequently involved.

Of the thirty-one subjects in whom splinter haemorrhages were present, only four could give a definite history of trauma to the hands. In three of these, the trauma had occurred during recreational activities (cricket, home-decorating and camping) and in one at work (a park-keeper). In the remaining twenty-seven, no history of trauma could be elicited.

DISCUSSION

Interpretation of the significance of splinter haemorrhages in patients depends upon a knowledge of their prevalence in healthy subjects. There are only two previous reported studies. Platts & Greaves (1958) found none in thirty-five individuals of unstated age and sex. However, their study excluded an unknown number whose splinter haemorrhages were discounted on the grounds of a history of recent trauma. Robertson & Braune (1974) found twenty-two in forty females (55%) and thirteen in twenty-four males (54%). The prevalence found in their study is remarkably high when compared with that found in two large series of hospital in-patients, 18% of 429 subjects (Kilpatrick *et al.*, 1965) and 19·1 % of 267 (Gross & Tall, 1963).

In the present study, splinter haemorrhages were found sufficiently commonly in healthy males to severely limit their value as a clinical sign of systemic disease. In healthy females, however, they were found substantially less frequently, and when present were confined to a single digit in all instances. It would therefore be reasonable to conclude that in females the presence of splinter haemorrhages on more than one digit should raise the suspicion of an underlying pathological disorder.

There is no evidence from the literature as to whether the location of splinter haemorrhages on the nail (i.e. whether proximal or distal) relates to the presence or absence of an underlying systemic disorder. This was not investigated in this study.

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Hyperpigmentation of the face and hands without blisters: porphyria cutanea tarda

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SUMMARY

Hyperpigmentation of the face and hands without blisters and hypertrichosis in Ethiopian patients with liver disease represents an early or latent form of porphyria cutanea tarda.

In Ethiopia, darkening of the face is a common clinical feature of liver disease which in turn accounts for about 12% of hospital admissions (Tsega, 1976; Lester, 1978). The cause of this striking hyperpigmentation in the absence of the other signs of porphyria cutanea tarda (PCT) is not known, although the latter is common among drinkers of local alcoholic beverages.

A total of twenty-five patients with hyperpigmentation of the face and hands but without blisters were seen and investigated between March 1977 and May 1979, during a study of patients with PCT. Most of these patients were referred to Black Lion Hospital because of suspicion of liver disease. The results are compared to those of patients with overt PCT and normal controls.

MATERIAL AND METHODS

Patients and controls

There were twenty-five patients with hyperpigmentation of the face and hands (twenty-three men and two women), eighty-six patients with confirmed PCT and twenty-six normal controls. All groups were comparable in age and sex. They were interviewed and examined with emphasis on occupation, exposure to sunlight, alcohol and drug ingestion and history of jaundice or liver disease. Freshly voided urine was allowed to stand in a beaker for 1–2 h and the colour recorded.

Biochemical studies

Blood was drawn from patients and volunteer controls for serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase, total and direct serum bilirubin, bromsulphalein (BSP), serum protein electrophoresis and prothrombin time (determined as Quick test), serum iron and total iron binding capacity (TIBC). Aliquots from 24-h

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urine collections from patients and controls were kept at −20°C until isolation and assays of porphyrins and their precursors were made by the methods of Mauzerall & Granick (1956), Fernandez, Henry & Godenberg (1966) and Jacobs (1974).

Liver and skin biopsy

Eleven of the hyperpigmented patients without blisters and forty-eight with PCT had a successful percutaneous liver biopsy using a Menghini needle. The liver tissue was studied for parenchymal changes and siderosis with haematoxylin and eosin stains as well as by Perls' technique for iron. Six of each group of patients and three of the volunteer controls had skin punch biopsies over a specified area of the dorsum of the left hand to study structural changes, melanocyte density and the degree of iron deposition. Skin sections were stained with periodic acid Schiff, and by Schmorl's method for melanin. Auto-fluorescence of porphyrin pigments in both liver and skin tissues was studied in a dark room under a binocular dissecting microscope, illuminated by a 110 W UVL inspection lamp (Allen) designed for engineering inspection. This examination must be made within a few hours of obtaining the biopsy, as the fluorochrome diffuses out into the fixative.

RESULTS

Patients and controls

The majority of patients and controls came from Addis Ababa and its vicinity. Patients with hyperpigmentation alone consumed significantly less alcohol than those with PCT. The degree of exposure to sunlight was the same in both. Unlike the patients with PCT, all but three of the hyperpigmented group showed no reddening of standing urine. While there was only one patient with a history of jaundice and three with hepatomegaly among the hyperpigmented, there were seven with a history of jaundice and six with hepatosplenomegaly among those with PCT. Hyperpigmentation is a common feature of both but hypertrichosis was seen only in patients with PCT.

Biochemical studies

The serum transaminases and BSP were elevated in both groups except in two of the hyperpigmented patients without blisters. The serum alkaline phosphatase, bilirubin, albumin and globulin were normal in both. None of the controls had abnormal liver function test results. The urinary uroporphyrin and coproporphyrin levels were significantly elevated in fourteen of the patients with hyperpigmentation alone and in all with PCT. In eleven of the hyperpigmented patients and the normal controls neither uroporphyrin nor coporporphyrin was elevated. Both the serum iron and TIBC levels were variable in each group.

Histopathology

Liver. Of the eleven patients with hyperpigmentation who had a liver biopsy, four had macronodular cirrhosis (one with hepatoma) and the rest had variable degrees of inflammation and fibrosis. Six of the liver biopsies revealed marked siderosis and three of these showed fluorescence of porphyrin pigments under the UVL. Those with contraindications to biopsy (fourteen patients) were clinically and biochemically diagnosed as having liver disease of undetermined type except three who had neither clinical nor biochemical evidence. Liver biopsy in forty-eight of the eighty-four patients with PCT revealed inflammation and fibrosis in thirty-four, inflammation only in five, fibrosis only in one, cirrhosis in six and primary hepatocellular carcinoma with macronodular cirrhosis in two. Forty-four of these patients showed above normal hepatic siderosis while fluorescent porphyrin under UVL was detected in thirty-one only.

Skin. None of the skin biopsy specimens from the six hyperpigmented patients without blisters, the six with PCT, and the three controls showed fluorescence of porphyrin under UVL, or a significant amount of iron. Melanin density was moderate to intense in all but one of the hyperpigmented patients without blisters, slight to moderate in all six patients with PCT and slight in one but intense in two of the controls. One of the hyperpigmented patients without blisters and three of the patients with PCT revealed separation of the dermo–epidermal junction. This is the histological equivalent of blister formation in the earliest stages and suggestive of PCT. The subpapillary plexus was dilated in two of the hyperpigmented and four of the PCT groups but no excessive polysaccharide was demonstrable in the wall using the periodic acid Schiff stain.

TABLE 1. Clinical, biochemical and histopathological comparison of patients with hyperpigmentation without blisters, PCT and controls

	Patients and controls		
	Hyperpigmented only	PCT	Controls
Clinical			
Origin	Mostly from Addis Ababa	Mostly from Addis Ababa	Mostly from Addis Ababa
History of liver disease	Primary presentation	Secondary presentation	None
Alcohol consumption	+ +	+ + + +	0–+
Exposure to sun	+ +	+ +	+
Reddening of standing urine	3/25 (12%)	78/78 (100%)	—
Blisters on hands	None	78/78 (100%)	None
Hypertrichosis	None	78/78 (100%)	None
Biochemical			
Transaminases BSP elevation	23/25 (92%)	78/78 (100%)	Normal
Uroporphyrin Coproporphyrin elevation	14/25 (56%)	78/78 (100%)	Normal
Serum Fe and TIBC	Variable	Variable	Not done
Histopathology			
(a) Liver biopsy			
Abnormal histology	11/11 (100%)	48/48 (100%)	Not done
Increased hepatic siderosis	6/11 (55%)	44/48 (92%)	Not done
Fluorescing porphyrin pigment	3/11 (27%)	31/48 (66%)	Not done
(b) Skin biopsy			
Iron, porphyrin pigment	None	None	None
Melanin density	+ + + +	+ +	+ + +
Dermo–epidermal separation	1/6 (17%)	3/6 (50%)	Normal

DISCUSSION

The clinical, biochemical and histological results in both groups of patients and controls are summarized in Table 1. Although there are several factors influencing melanin density (such as the basic skin colour of the individual, the effect of recurrent blisters leaving behind hypopigmented scars in those with PCT, degree of exposure to sunlight, etc), all of the hyperpigmented patients without blisters had moderate to intense melanin density. The dermo–epidermal junction was separated in four patients, one of whom was from the hyperpigmented group. Similarly, the subpapillary plexus was dilated only in six of the patients, four of whom had PCT.

Some of the clinical, biochemical and histological findings in the hyperpigmented group merge into those with PCT, the only difference being the absence of blisters in the former. These observation suggest that the hyperpigmented group of patients without blisters represent early or latent forms of PCT. What triggers the formation of blisters in some but not in others, especially in the hyperpigmented patients with all the features of PCT, remains unknown. The early hepatic parenchymal changes associated with elevated transaminases but normal serum protein electrophoresis, are unlikely to account for delayed break-down of melanin stimulating hormone and hence for the hyperpigmentation in these patients. Hyperpigmentation of the skin may be due to photosensitivity to porphyrin in amounts undetectable by the methods employed in this study (Malina, Miller & Magnus, 1978).

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Pharmacology and Treatment

Detection of the action of salicylic acid on the normal stratum corneum

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SUMMARY

The undoubted clinical usefulness of salicylic acid contrasts markedly with its barely detectable action on normal skin. In this study we have attempted to extend our understanding of the action of salicylic acid by monitoring its effect on the stratum corneum using newly developed techniques for assessing desquamation, as well as using conventional methods for measuring other aspects of epidermal activity.

Our findings have succeeded in demonstrating that salicylic acid enhances the shedding of corneocytes and we suggest that this compound in some way decreases corneocyte to corneocyte cohesion.

We have confirmed that salicylic acid has no effect on the mitotic activity of the normal epidermis.

Salicylic acid (SA) is used extensively in a wide variety of skin disorders in which there is an abnormality of the stratum corneum leading to scaling or hyperkeratosis. Despite its undoubted and often dramatic clinical effectiveness, it has proved difficult to demonstrate its action on normal skin (Davies & Marks, 1976; Huber & Christophers, 1977). In this study we have attempted to extend our understanding of SA by monitoring its effect on the stratum corneum, using newly-developed techniques for measuring desquamation, as well as measuring stratum corneum thickness and epidermal mitotic activity.

MATERIALS AND METHODS

Experimental 1

In five volunteers (mean age 49 years; two males, three females) without generalized skin disorders or systemic disease, 'forced' desquamation corneocyte counts were obtained using a hand held scrub apparatus (Roberts & Marks, 1979; Guibarra 1978) over the first 8-h period after application of 6% SA in 70% alcohol to one forearm and 70% alcohol alone to the opposite forearm as a control. The samples were taken at a different site on the forearm on each occasion.

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Experiment 2

Six subjects (mean age 58 years; five females, one male) without generalized skin disorders or systemic disease participated in the study after giving their informed consent. Six per cent SA in 70% alcohol was applied twice daily to the flexor aspect of one forearm for 10 days, and 70% alcohol alone was applied twice daily to the other forearm as a control.

'Forced' desquamation using the hand held scrub apparatus was measured on both sites, on a separate area on each occasion, on alternate days for 10 days. Prior to the initial application of the test materials, small portions (1 cm^2) of the treated skin areas were stained with 1% silver nitrate solution (aqueous). The silver nitrate was 'reduced' with a photographic developer (Metol hydroquinone) and the resulting grey-black area photographed every 2 h each day until disappearance of the abnormal colour. The density of the stain on the photographic negative was measured using a densitometer and was expressed as the ratio of the density on stained to that on unstained skin (Roberts & Marks, 1980).

Twenty-four hours prior to application of the test materials, 5% dansyl chloride in white soft paraffin was applied to each forearm under occlusion (Jansen, Hojyo-Tomoto & Kligman, 1974). The treated areas were examined daily with a hand held ultraviolet lamp emitting primarily in the UV-A region and the number of days taken to the disappearance of fluorescence was recorded for each site. This was regarded as the 'turnover times' of the stratum corneum for the different sites examined. Four millimetre punch biopsy specimens were taken from treated and control sites at the end of the trial period. The biopsy specimens were divided in two, and one-half was immediately frozen in liquid nitrogen and sectioned on a cryostat at 12 μM prior to treatment with a solution of 0·5% methylene blue in order to visualise the stratum corneum. The numbers of cell layers of the stratum corneum in each specimen were counted at ten different sites and the mean of these was taken as the thickness of the horny layer. The remainder of each specimen was sliced into 1 mm thick slices and incubated in Eagle's medium, containing tritiated thymidine (1 μc/ml, specific activity 18 Cl/m mol) at 37°C for 2 h. Autoradiographs were obtained by standard methods (Marks & Wells, 1973) and the number of labelled basal and suprabasal epidermal cells recorded (a cell with more than six grains over background per nucleus was scored as labelled). The results were then expressed as a percentage of labelled cells to the total number of basal cells (labelling index).

RESULTS

The 'forced' desquamation cell counts over the initial 8-h period in Experiment 1 are shown in Table 1. The cell counts from the SA treated area increased slightly as compared to those from the control arms in the 2–3 h period and then declined. There was little change in the cell counts at the sites treated with 70% alcohol alone.

The 'forced' desquamation cell counts over the 10-day test period (Experiment 2) are shown in Fig. 1. With the SA solution there was a marked persistent fall in the number of cells released. With the 70% alcohol solution there was an initial marked fall in the corneocyte counts, which rose again to normal levels after 4 days. The differences between the corneocyte counts using SA and 70% alcohol solution are statistically significant on days 6, 8 and 10 ($P<0·02$).

The results of the rate of loss of fluorescence due to dansyl chloride are shown in Table 2. There were no significant differences between the test and control groups.

The results of the silver nitrate photographic densitometry technique are represented in Fig. 2. The stain tended to be released more rapidly from the treated sites than from the control sites; however, the differences were not significant.

The cryostat sections treated with methylene blue solution demonstrated differences between test

TABLE 1. Number of corneocytes released (cells/cm^2/10 s × 10^3) by scrub technique over initial 8 h after application of 6% salicylic acid in 70% alcohol and 70% alcohol alone

	Salicylic acid in 70% alcohol						70% Alcohol					
Time (h)	1	2	3	4	5	Mean ± s.d.	1	2	3	4	5	Mean ± s.d.
0	102	76	140	115	51	96·8 ±34·5	115	89	121	127	51	100·6 ±31·3
1	115	76	127	140	64	104·4 ±32·9	102	64	134	127	51	95·6 ±37·0
2	153	102	140	166	102	132·6 ±29·4	115	89	127	115	51	99·4 ±30·4
3	153	115	153	153	127	140·2 ±16·1	127	76	108	134	64	101·8 ±30·9
4	146	89	140	166	115	131·2 ±26·6	102	102	127	115	89	107·0 ±14·5
6	115	76	115	153	89	109·6 ±29·6	102	76	153	140	64	107·0 ±38·9
8	115	89	127	127	102	112·0 ±16·5	108	89	102	153	95	109·4 ±25·4

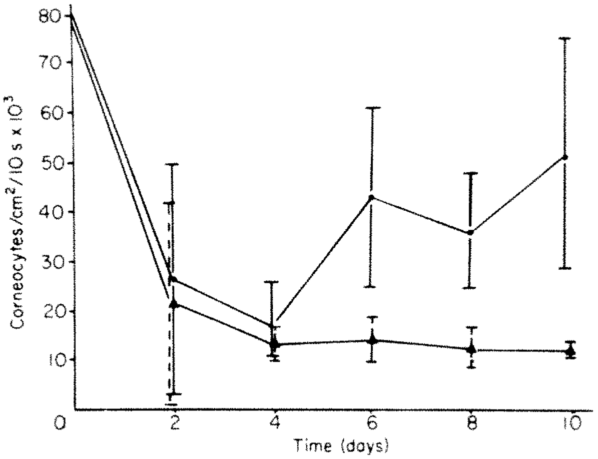

FIGURE 1. Number of corneocytes released using scrub apparatus (10 s) on alternate days. (●——●) 70% alcohol; (▲——▲) 6% salicylic acid in 70% alcohol.

and control specimens. Specimens from the SA treated sites had a much thinner stratum corneum than the control biopsies ($P < 0{\cdot}05$). The mean thicknesses of the horny layer in number of cells of test and control biopsies are represented in Table 3.

The labelling indices of autoradiographs derived from tissue incubated with tritiated thymidine are given in Table 4. As can be seen, there was no significant difference between the test and control groups.

TABLE 2. Days to disappearance of fluorescence with dansyl chloride

Subject	6% Salicylic acid in 70% alcohol	70% Alcohol alone
1	20	18
2	15	12
3	12	12
4	23	20
5	11	11
6	15	18
Mean	16	15·2
S.d.	4·6	3·9

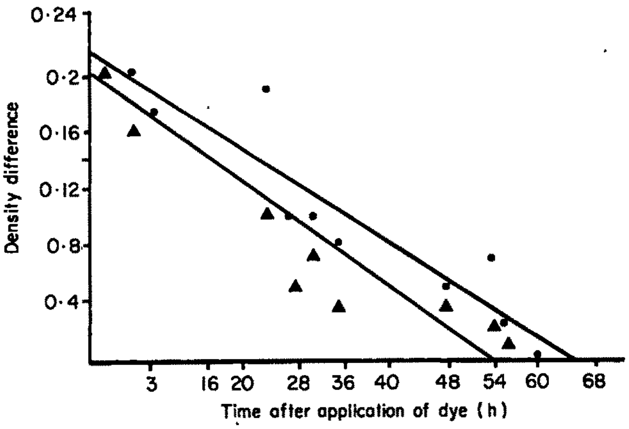

FIGURE 2. Density difference of stained and unstained skin. (●) 70% alcohol; r = 0·924; (▲) 6% salicylic acid in 70% alcohol; r = 0·913.

TABLE 3. Mean epidermal thickness in number of cell layers before treatment and after applying test and control preparations for 10 days

Subject	Before treatment	Salicylic acid	70% Alcohol
1	24	18	26
2	26	16	24
3	20	16	18
4	22	18	20
5	26	14	20
6	22	15	24
Mean	23·3	16·1	22
S.d.	2·4	1·6	3·1

TABLE 4. Labelling indices after applying test and control preparations (% basal and supra-basal cells labelled of total number of basal cells)

Subject	Salicylic acid	70% Alcohol
1	7·2	8·1
2	6·8	7·5
3	9·3	7·5
4	5·7	6·1
5	7·9	8·5
6	6·0	5·8
Mean	7·15	7·25
S.d.	1·3	1·1

DISCUSSION

Salicylic acid has an important part to play in the treatment of many disorders in which there is scaling and hyperkeratosis. However, its mode of action has remained obscure. In part our ignorance has stemmed from the lack of techniques with which to monitor its effects and, in this investigation, we have attempted to employ the appropriate tests to rectify the situation.

We found that the rate of loss of silver nitrate from the surface of the horny layer was slightly faster with the SA treatment, suggesting an enhanced loss of corneocytes during the treatment period. This could not be detected using the dansyl chloride fluorescence technique, presumably because this method is more suitable for determining the gross turnover of the stratum corneum and is insufficiently sensitive in the short term.

It is interesting to note that over the initial 8 h (in Experiment 1) after treatment with SA solution corneocytes were easily displaced from the horny layer resulting in increased cell counts with the 'forced' desquamation technique. However, over a period of 10 days (Experiment 2) the number of corneocytes released decreased markedly with both preparations but remained at a low level with the SA solution. This persistently low cell count suggests that SA allows more corneocytes to be shed as a result of normal wear and tear than is usual, leaving the more adherent corneocytes behind. This is also supported by the decreased stratum corneum thickness found in the SA treated skin. We have confirmed that SA has no significant effect on the mitotic activity of the normal epidermis over a 10-day period.

Our findings, therefore, have succeeded in demonstrating that SA enhances the shedding of corneocytes and suggests that this compound in some way decreases corneocyte to corneocyte cohesion. It should be noted that although in the undisturbed horny layer there is a good correlation between forced desquamation and the passive loss of corneocytes as determined by a chamber technique (Roberts & Marks, 1980), this relationship may be disturbed in situations where corneocyte to corneocyte cohesion is altered near the surface due to the ease of detachability resulting from environmental friction. The findings reported above are also consistent with the results obtained by Huber & Christophers (1977) in guinea-pig skin *in vitro* and with a previous study of ours in which an alteration in stratum corneum arrangement and decrease in its thickness were found in SA treated skin (Davies & Marks, 1976).

The 'mystery' of a pronounced effect on abnormal stratum corneum in the face of barely detectable action on the normal stratum corneum may be explained as follows. The abnormal horny layer (regardless of the cause) exhibits failure of release of cohesion between corneocytes (Marks, 1979). If the action of SA is to decrease the 'stickiness' or cohesion, then this effect will be more noticeable when the abnormal cohesion is more pronounced.

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Case Reports

Lichen sclerosus et atrophicus of the scalp

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SUMMARY

A patient is described who developed pruritic lesions of the scalp with increasing hair loss and plaque formation over a period of 15 years, in association with patches if white atrophic skin on her trunk and vulva in the last 5 years. Biopsies of the scalp and trunk lesions both showed lichen sclerosus et atrophicus.

This would appear to be the first recorded case of lichen sclerosus et atrophicus of the scalp in the English literature.

CASE REPORT

A 71-year-old female of Scottish ancestry, who has lived in Australia for the previous 30 years, developed pruritus on the front of her scalp in 1964 with subsequent increasing hair loss. White patches developed in the areas of hair loss. This has progressed over the years to involve almost the complete scalp apart from the temporal regions. Since 1975 she has been aware of asymptomatic white patches appearing over her upper back and sternum. She gives a history of a 'warty lesion' on the left frontal region of her scalp which was excised and grafted in Australia in 1976. The histology of this lesion is not known.

Apart from a thyroidectomy in 1974 for thyrotoxicosis, she has had no other serious illnesses.

Examination revealed an extensive lesion with apparent dermal atrophy and focal hyperkeratosis covering 80% of her scalp (Fig. 1). The clinical differential diagnosis included chronic discoid lupus erythematosus, an infiltrating sclerotic basal cell carcinoma, or extensive actinic damage. However, an additional finding of interest was the presence of multiple areas of light violaceous atrophic skin over her sternum, upper back and vulva. Her facial appearance suggested marked solar elastosis although there was no evidence of actinic keratoses. The remainder of the physical examination was normal.

Routine blood biochemistry and haematology were normal, and serum thyroxine indicated an adequate level of replacement. A biopsy of the occipital region of the scalp (Fig. 2) showed marked epidermal atrophy, relative hyperkeratosis, oedema and hyalinization of the papillary dermis, with

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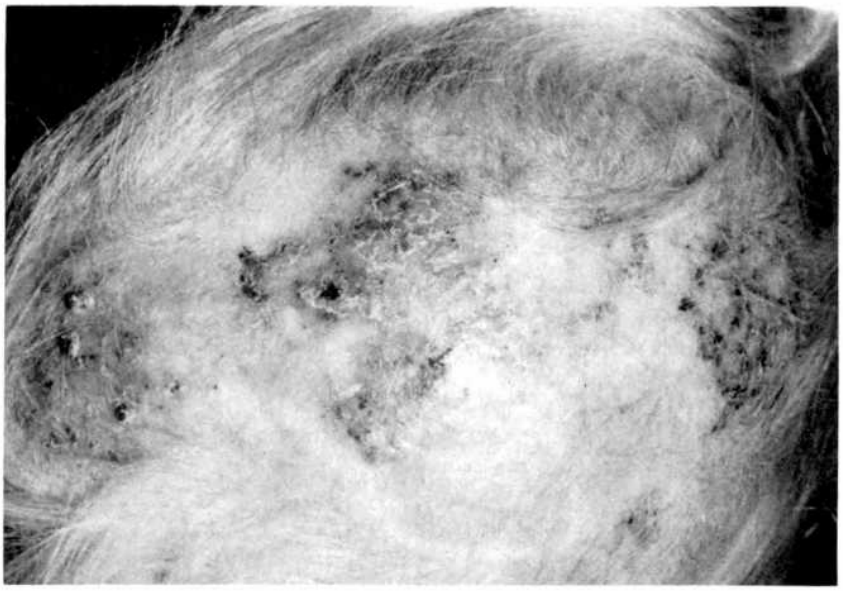

FIGURE 1. Scalp showing extensive changes with apparent dermal atrophy and focal hyperkeratosis.

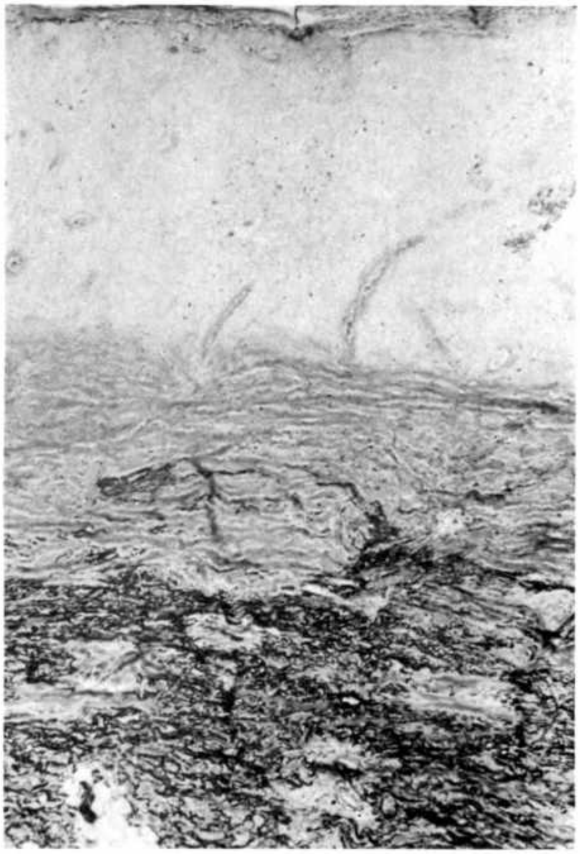

FIGURE 2. Histology of scalp lesion (Weigert's stain for elastica) showing total absence of elastic tissue in the upper layers of the dermis. Other diagnostic features are epidermal atrophy, loss of skin appendages, and hyalinization and oedema of the collagen of the papillary dermis (× 108).

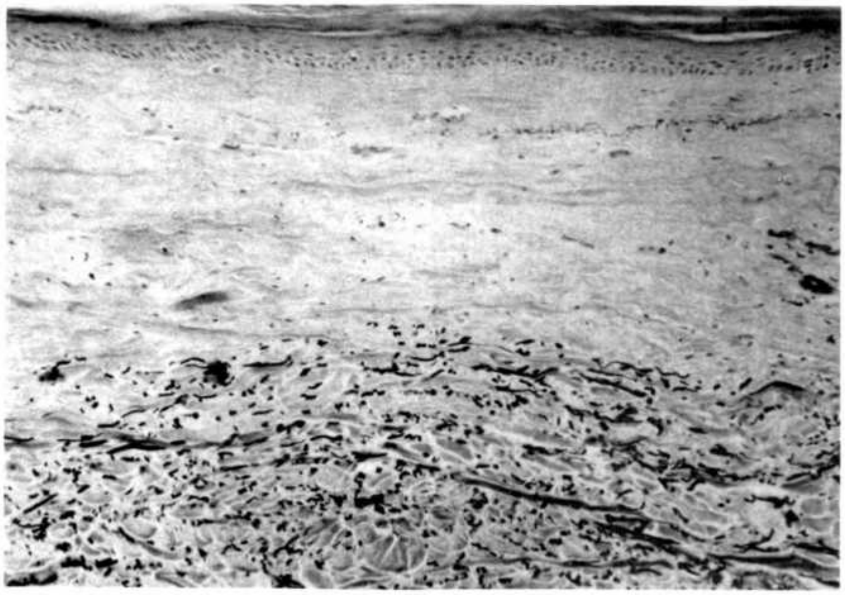

FIGURE 3. Histology of skin biopsy from lesion of upper back showing features similar to Fig. 2. (Weigert's elastica, × 270).

lymphocyte infiltration of the mid-dermis. This was comparable with a biopsy of one of the atrophic areas of the upper back (Fig. 3). Both biopsies were diagnostic of lichen sclerosus et atrophicus.

DISCUSSION

Lichen sclerosus et atrophicus was first described by Hallopeau in 1887, who considered the disorder to be a type of lichen planus. Nowadays most people regard it to be a separate entity. The condition is uncommon with females predominating (Nicolau & Bolus, 1966). The non-genital lesions are usually symptomless and occur particularly on the upper trunk and neck, around the umbilicus, and on the upper extremities. Occasionally lesions of the mouth or tongue occur (Rowell, 1972). Lichen sclerosus et atrophicus has been reported in several members of one family (Barker & Gross, 1962; Wallace, 1971).

In an extensive search of the English, French and German literature, the finding of lichen sclerosus et atrophicus of the scalp has not previously been reported, although it is well recognized to occur in sites other than the external genitalia (Wallace, 1971). Interestingly, the area of skin grafting of this patient's scalp resembled the remainder of the scalp, which compares with the findings of Whimster (1965) that normal skin transplanted onto a vulvectomy previously affected with lichen sclerosus et atrophicus could subsequently develop lichen sclerosus et atrophicus. This patient had had a previous thyroidectomy and it is now recognised that patients with lichen sclerosus et atrophicus have an increased incidence of auto-immune diseases (Harrington, 1979).

Less than 5% of patients with vulval lichen sclerosus et atrophicus develop carcinoma of the vulva (Wallace, 1971). It is generally recognized that the non-genital lesions should not be treated as they do not undergo malignant change. However, in view of the extensive nature of this patient's lesions it will be necessary to keep the patient under close supervision.

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Bullous pemphigoid, polymyalgia rheumatica and thyroid disease

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SUMMARY

This paper describes the occurrence of bullous pemphigoid in a 71-year-old female patient who subsequently developed polymyalgia rheumatica and hyperthyroidism. The association of bullous pemphigoid with other autoimmune disorders is discussed.

CASE REPORT

A 71-year-old woman was seen in October 1973 with a 4-month history of recurrent episodes of blisters involving the trunk and limbs. The lesions started as small itchy red lumps which subsequently formed blisters. Examination revealed intact firm bullae on an erythematous base over the abdomen and the flexor aspects of the limbs. A clinical diagnosis of bullous pemphigoid was made and biopsy of the abdominal lesions showed the histological features of sub-epidermal bullae. The haemoglobin level was 13·2 g/dl and the erythrocyte sedimentation rate (ESR) was 29 mm in the first hour. Treatment with prednisone 30 mg daily led to improvement of the skin lesions, which were controlled thereafter on a maintenance dose of 5 mg prednisone daily.

In April 1974, while still on prednisone 5 mg daily and free from skin lesions, she was referred with a 6-week history of increasing pain and stiffness affecting the neck, back, shoulder and pelvic girdle muscles. The stiffness was particularly noticeable in the morning when she would experience considerable difficulty in getting out of bed. There was no history of headaches or visual disturbance. On examination there was limitation of movement of the shoulders, hips, knees and neck but no joints showed signs of inflammation. The haemoglobin level was 11·0 g/dl and the ESR was 80 mm in the first hour. Rose-Waaler agglutination and RA latex tests were negative, and radiological examination of the joints was normal except for minor osteoarthritic changes. Protein electrophoresis showed an increase in the alpha-2 fraction. Liver function tests and serum creatine phosphokinase were normal. A diagnosis of polymyalgia rheumatica was made, and the patient was maintained on

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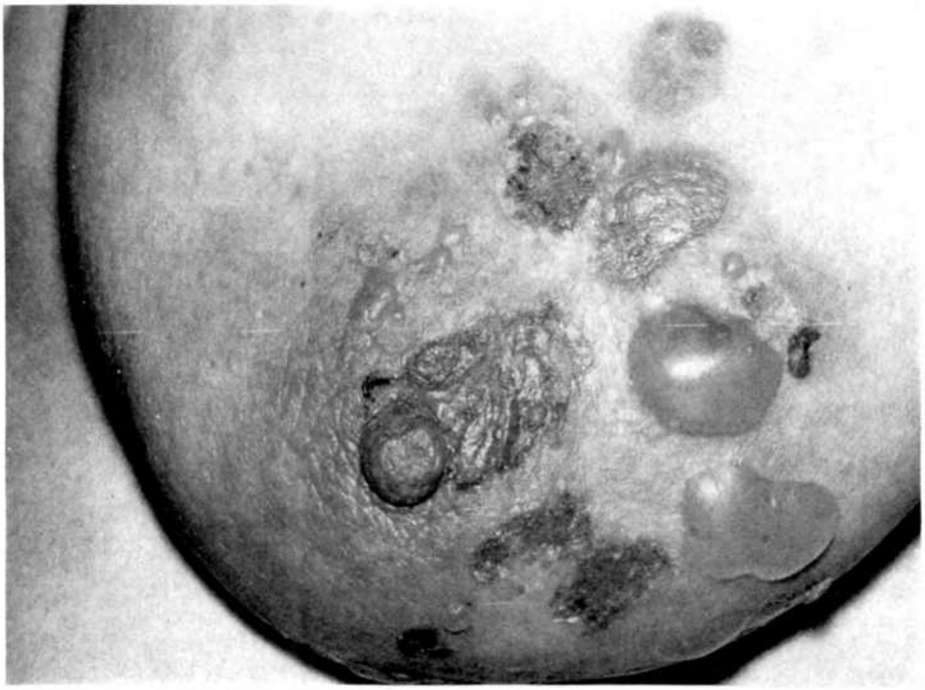

FIGURE 1. Bullous lesions on breast.

prednisone 5 mg daily but in addition she received Benoral suspension orally 10 ml twice daily and local injections of Depo-medrone. There was some improvement but 1 month later she was once again troubled with pain and stiffness in the shoulder and pelvic girdle. The ESR remained elevated at 70 mm in the first hour. Unfortunately she was lost from follow-up and was not seen again until October 1974. The patient had apparently stopped the prednisone in late July 1974 and was now experiencing minimal pain and stiffness. The ESR at this stage was 27 mm in the first hour.

In April 1975, she was referred back to the Department of Dermatology with a 3-week history of recurrence of the blisters. Examination revealed the presence of intact firm bullae on an erythematous base on the abdomen and flexor aspects of the limbs as in 1973, but in addition similar lesions were now present on the breasts (Fig. 1). Direct immunofluorescent studies were undertaken which showed the presence of IgG and complement in the basement membrane, in keeping with the diagnosis of bullous pemphigoid. The patient was treated with prednisolone 30 mg daily but there was only moderate improvement and azathioprine 150 mg daily was started. The subsequent course was satisfactory and it was possible to stop the prednisolone and azathioprine towards the end of 1975.

In September 1977, the patient was referred to the Thyroid Clinic with a 3-month history of tiredness, irritability, excessive sweating, heat intolerance and weight loss of 7 kg. She was clinically hyperthyroid and had a diffuse moderately enlarged thyroid gland, lid lag and lid retraction. The diagnosis of hyperthyroidism was confirmed by the findings of an elevated serum thyroxine level (223 nmol/l) and an increased 4 h^{132}I thyroid uptake test (39%). Tests for anti-thyroglobulin and anti-thyroid microsomal antibodies were negative. She was treated with radioiodine (10 mCi of ^{131}I) and was clinically and biochemically euthyroid two months later. However, in March 1978, she became hypothyroid and since then has been on thyroxine replacement therapy.

There was no family history of autoimmune disorders except for the patient's late mother who had suffered from rheumatoid arthritis.

DISCUSSION

Pemphigoid is a chronic bullous disease affecting primarily middle-aged and older patients. Its aetiology is unknown although an immunological basis has been suggested by the serological finding of IgG antibodies against skin basement membrane in approximately 80% of cases (Provost, 1978). However, there is so far little evidence to implicate these antibodies as causing the lesions, and it is of interest that some individuals may have such circulating antibodies but no signs of skin disease (Champion & Parish, 1975). It has been suggested that participation of IgE antibodies against basement membrane in addition to the IgG may be necessary to induce lesions (Champion & Parish, 1975).

One of the hallmarks of autoimmune disorders is their tendency to co-exist in the same individual (Roitt, 1977), and observations of the involvement of pemphigoid in such associations would therefore lend support to the view that pemphigoid itself may be an autoimmune disease. Such observations have been infrequently described, but there are reports of cases of pemphigoid co-existing with rheumatoid arthritis (Lillicrap, 1963; Salo & Räsänen, 1972; Cullen *et al.*, 1978), systemic lupus erythematosus (Jordan *et al.*, 1969; Miller, Downham & Chapel, 1978), polymyositis (Peck & Lefkovits, 1966) and pernicious anaemia (Muller *et al.*, 1971; Obasi & Savin, 1977). More recently, Hamilton & McKenzie (1978) described the simultaneous occurrence of bullous pemphigoid and primary biliary cirrhosis in a 70-year-old man who was also found to have vitiligo. In addition, there are a few examples in the literature of the co-existence of bullous pemphigoid with other skin diseases of a putative autoimmune nature, such as dermatitis herpetiformis (Honeyman *et al.*, 1972), pemphigus vulgaris (Chorelski *et al.*, 1974) and pemphigus foliaceus (Harrington & Sneddon, 1979).

Polymyalgia rheumatica is closely related to giant cell (temporal) arteritis, and it is now generally considered that these two syndromes result from the same underlying arteritis (Alestig & Barr, 1963; Hamrin, Jonsson & Lanberg, 1964; Dixon *et al.*, 1966; Wilske & Healey, 1967; Mowat & Hazleman, 1974). There is also some evidence to suggest that polymyalgia rheumatica and giant cell arteritis may have an immunological basis (Liang, Simkin & Mannik, 1974; Hazleman, MacLennan & Esiri, 1975; Waaler, Tonder & Milde, 1976; Eghtedari, Esselinck & Bacon, 1976; Malmvall *et al.*, 1976) and in this respect it is of interest that these syndromes have been reported in association with auto-immune thyroid disease (Thomas & Croft, 1974; How, Bewsher & Walker, 1977; Dent & Edwards, 1978) and primary biliary cirrhosis (Robertson, Batstone, & Leobl, 1978). The present case is a further example of the association of polymyalgia rheumatica with thyroid disease but in addition the patient had bullous pemphigoid, and it would appear that this triad in one individual has not been previously reported. However, Hamilton & McKenzie (1978) quoted that two out of twenty patients with bullous pemphigoid were found to have antithyroid antibodies, one of whom subsequently developed myxoedema, while in an earlier report Muller *et al.* (1971) had noted two patients with pemphigoid and auto-antibodies against gastric parietal cells and thyroid cytoplasm.

There is a need for a careful prospective study of a large number of pemphigoid patients to establish whether there is a significant association with other autoimmune disorders.

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Unusual presentation of extragenital cutaneous Schistosomiasis mansoni

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SUMMARY

An African patient with a hypopigmented plaque on the face, suggesting clinically tuberculoid leprosy or sarcoidosis, is described. Histology revealed palisading granulomas surrounding ova of *Schistosoma mansoni.*

While genital and perineal schistosomal granulomata are not uncommon, extragenital cutaneous lesions, particularly those caused by *Schistosoma mansoni*, are rare (Findlay & Whiting, 1971; Convit & Reyes, 1973; Wood, Srolowitz & Schetman, 1976; MacDonald & Morrison, 1976; Torres, 1976; Piva, Correro & Diniz, 1978; Bittencourt *et al.*, 1979; Andrade, 1979).

The following case report describes an extragenital cutaneous lesion of *Schistosoma mansoni* of unusual morphology and location and appears to be the first from West Africa.

CASE REPORT

A 13-year-old Nigerian boy entered the Skin Clinic of ABU Hospital, Zaria, Nigeria on 13 March, 1979 with a 1-year history of a hypopigmented lesion on his face. Physical examination showed a single, large (12 × 3 cm), irregular but sharply delineated plaque on his forehead and right temple (Fig. 1).

The lesion was hypopigmented, this being more pronounced at the margin of the plaque. The central part of the lesion was covered with fine branny scales and seemed to have regained some pigmentation. The whole lesion was at the same level and papular elements could not be found within the plaque. Sensation within the lesion was normal. The remainder of his skin was normal and he was otherwise in good health and well nourished. The clinical diagnosis was sarcoidosis, tuberculoid leprosy or pityriasis alba. Two 4 mm punch biopsies were taken from separate sites on the lesion. Both biopsy specimens showed essentially the same findings; the most striking feature was the presence in the dermis of schistosomal ova within palisading granulomas. Predominant eosinophils, less numerous neutrophils and histiocytes with occasional lymphocytes and giant cells formed a

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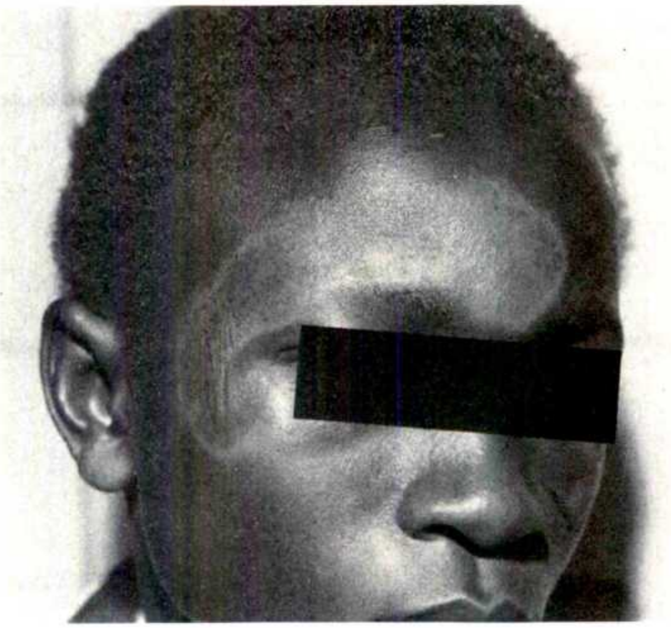

FIGURE 1. Hyperpigmented lesion on face of patient.

palisading zone around a central necrotic area containing ova, some of them completely or partially degenerated (Fig. 2). A few ova showed a lateral projection (Fig. 3). The morphology of the ova with the characteristic lateral spines was consistent with that of *Schistosoma mansoni*. The ova were stained only slightly by the Ziehl Neelsen method for acid-fast bacilli.

Further direct questioning when the results of skin biopsies were known revealed that the patient had had episodes of bloody diarrhoea. Repeated stool examinations were negative, but schistosomal

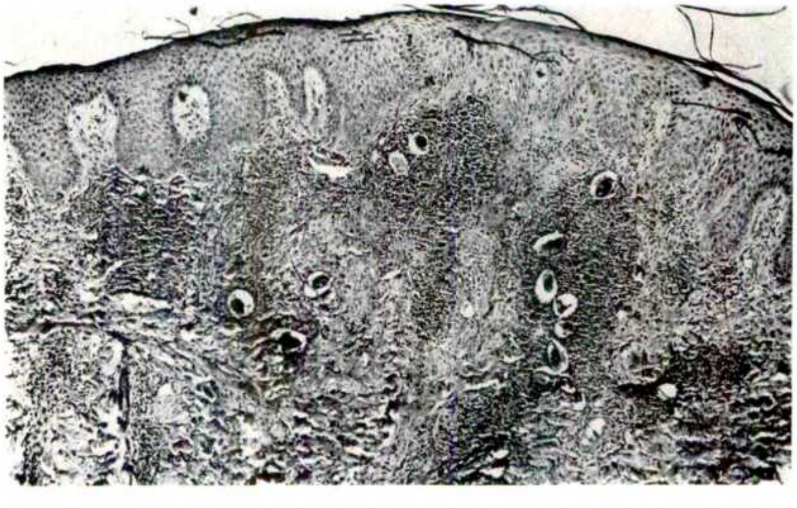

FIGURE 2. Section from the lesion showing multiple ova within granulomas (H&E, × 50).

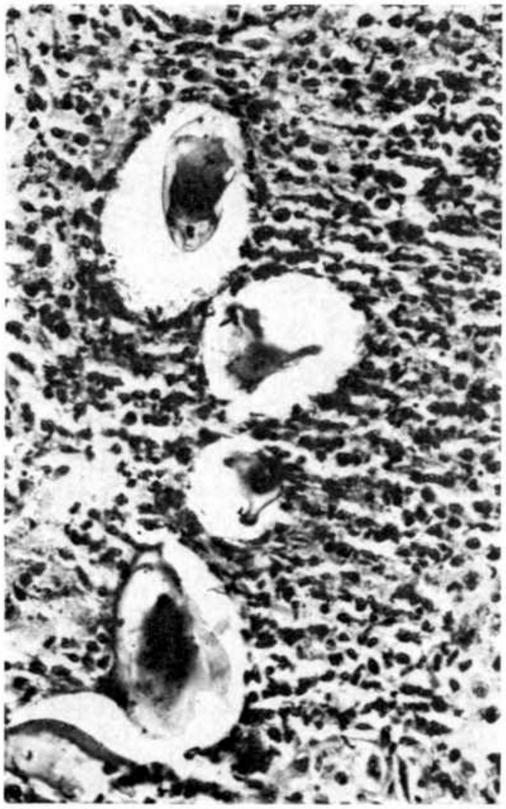

FIGURE 3. Ova of *Schistosoma mansoni* with lateral projections.

eggs were found in a rectal biopsy. An intradermal test with a Melcher type acid-soluble fraction of a buffered saline extract of adult *Schistosoma mansoni* (Wellcotest) gave a positive immediate reaction.

The patient was treated with Niridazole tablets (500 mg three times daily for 8 days). No evident change in his skin condition was noticed during three weeks of post-treatment observation. Later he defaulted from follow-up.

DISCUSSION

Ten well documented cases of extragenital and extra-anal cutaneous Schistosomiasis mansoni have been reported. Only two of these came from Africa (Findlay & Whiting, 1971; Wood, Srolowitz & Schetman, 1976). Interestingly, in both descriptions of patients infested in Africa, extragenital cutaneous lesions coincided with neurological manifestations attributed to the presence of *Schistosoma mansoni* eggs in the central nervous system.

The uniqueness of the present case lies in the clinical appearance and location of the extragenital lesion. In the majority of described cases of extragenital cutaneous Schistosomiasis mansoni, there were papular eruptions with a tendency to coalesce and form irregular plaques and the lesions were located on the trunk and in particular around the umbilicus. Similar morphology and distribution were also observed in cases of extragenital cutaneous Schistosomiasis haematobium (Cahill & El Mofty, 1964).

Papular lesions in a band-like zosteriform pattern observed in extragenital Schistosomiasis haematobium (Black 1946; Findlay & Whiting, 1971) have not been described so far in patients infested with *Schistosoma mansoni*. The only patient with a facial location of Schistosomiasis mansoni was reported by Andrade (1979). In this patient, an erythematous plaque was seen on the nose. No case of cutaneous schistosomiasis presenting as dyschromic, minimally infiltrated plaque simulating tuberculoid leprosy, sarcoidosis or pityriasis alba has been found in the literature. Asymptomatic deposition of Schistosoma ova in the skin of the face was reported as extremely rare in the large series of Ramos (1973) in Mozambique. The present case showed that in endemic areas the late extragenital skin manifestations of Schistosomiasis have to be considered in the differential diagnosis of cutaneous granulomas along with sarcoidosis, granuloma multiforme and tuberculoid leprosy.

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Herpetic arthritis associated with disseminate herpes simplex in a wrestler

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SUMMARY

A case of widespread inoculation herpes simplex of the skin is reported in a wrestler. Five days after the skin lesions appeared, the right knee became markedly tender and swollen. *Herpesvirus hominis* type I was cultured from the joint exudate as well as from the skin lesions. The determinant of the monoarticular localization of the arthritis appeared to be a prior injury to the knee, rendering it a specific site for deposition of the blood-borne virus.

The commonly recognized clinical locus for *Herpesvirus hominis* is the epidermis or mucosa. However, in widespread cutaneous eruptions a viraemic phase occurs, and encephalitis or hepatitis may ensue. The present report describes a previously undocumented element of such disseminate infection, viz. herpetic arthritis.

CASE REPORT

An 18-year-old college wrestler was admitted to the Hospital of the University of Pennsylvania for the treatment of an extensive crusting vesiculobullous eruption of the forearms, neck, forehead and scalp of 5 days duration (Fig. 1). Over fifty plaques were counted. On the day of admission his right knee became markedly tender, hot and swollen (Fig. 2). Minimal movement induced marked pain. An initial diagnosis of herpes gladiatorum with an associated arthritis was made.

He gave a past history of hay fever, an attack of herpes zoster at age 4, a severely wrenched right knee at age 11, and a sensitivity to sulphonamide drugs. He denied ever having had fever blisters or cold sores.

General physical examination on admission was normal, aside from the findings noted and some axillary adenopathy. His temperature was 99·6°F, and never exceeded this during our observation. Routine laboratory studies were within normal limits, including complete blood count, urinalysis and culture, sedimentation rate, ECG, chest X-ray, serological tests for syphilis, liver enzyme levels, and a serum protein electrophoresis. The blood showed a 1+ level of cryoglobulins and a herpes neutraliza-

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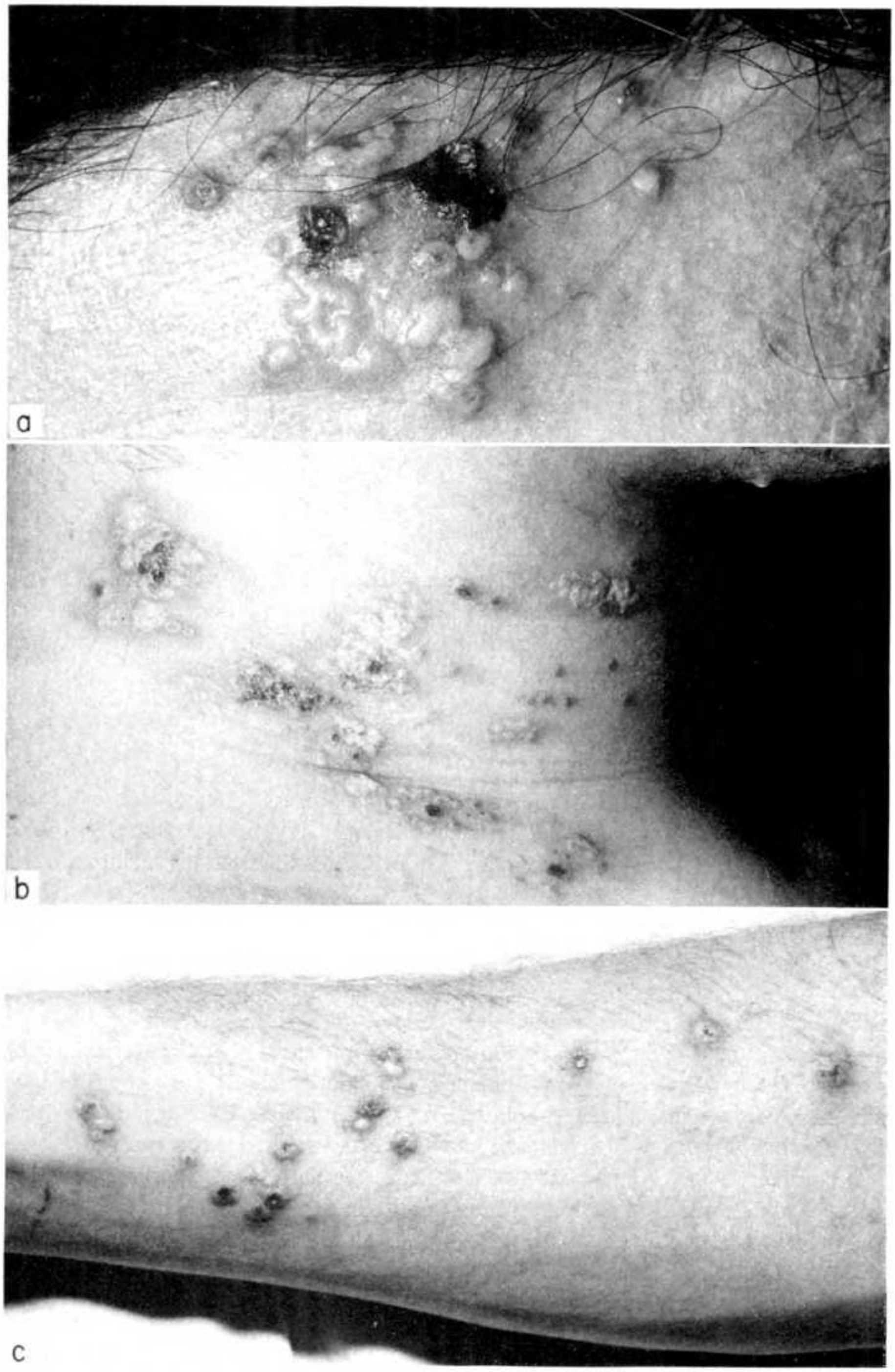

FIGURE 1. Inoculation herpes simplex lesion in (a) forehead, (b) neck, and (c) arm of patient. Note classical umbilication and clustering of lesions at sites of abrasive trauma during wrestling. HVH (Type I) was cultured from lesions on the forearm.

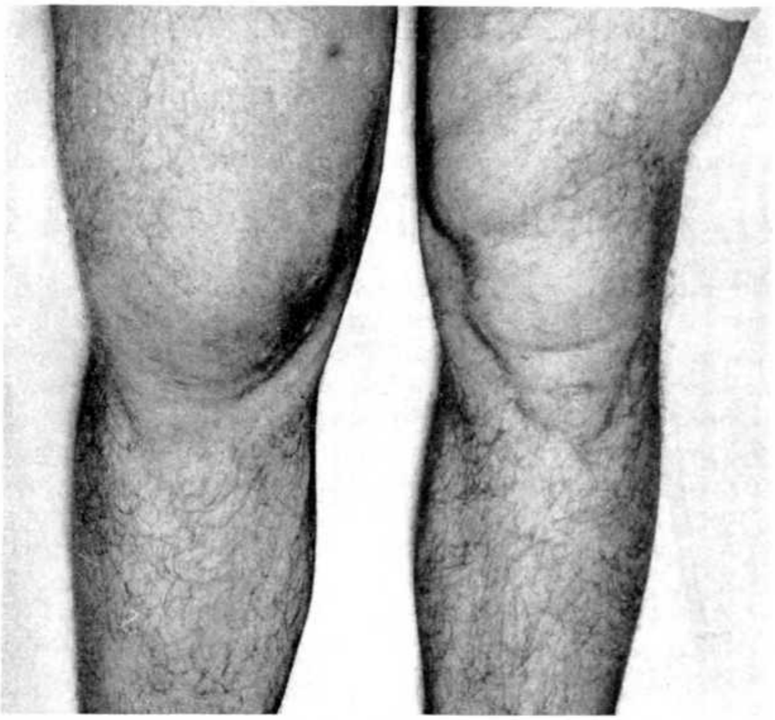

FIGURE 2. Inflamed swollen right knee which developed 5 days after disseminate cutaneous infection. Herpes virus (HVH Type I) was cultured from the aspirated joint fluid.

tion titre of less than 1/8. Ophthalmological examination revealed no herpetic lesions. Rheumatological consultation favoured a diagnosis of viral induced synovitis.

Culture of the vesiculopustular lesions on the right upper arm showed *Herpesvirus hominis* type I whereas culture for bacteria showed rare *Staphylococcus aureus* colonies. Bacteria were not seen on Gram stain. Culture of the effusion from the right knee showed *Herpesvirus hominis* Type I. The fluid showed normal viscosity, a good mucin clot formation, 10,000 WBC/mm^2 (80% mononuclear cells), and a negative Gram stain, and no bacteria were found on culture.

Biopsy of the skin lesion showed an intraepidermal acantholytic vesicle containing multinucleate giant cells suggestive of viral infection, necrosis of tissue and thickened proliferating capillaries, with swollen endothelial cells, as well as a perivascular infiltrate of lymphocytes and histiocytes. Biopsy of the synovium of the right knee revealed no inclusion bodies.

Treatment consisted of topical iododeoxyuridine, systemic erythromycin and bed rest.

The skin lesions gradually dried, crusted and involuted. An increased range of motion of the knee paralleled the decrease in swelling. The patient was discharged from the hospital after 5 days. The subsequent course was one of continuing improvement, so that he returned to full activities 2 weeks later. He learned that two of his wrestling colleagues had developed widespread herpes after having wrestled with him during the period just before his hospitalization. He has experienced no further lesions during the ensuing 10 months.

COMMENT

It is possible to reconstruct the pathogenesis of our patient's problem rather satisfactorily. Wrestling with an opponent who had an active herpes simplex of the face, our patient had the live virus (HVH Type I) rubbed directly into friction abraded skin of his arms, neck and face. The numerous inoculation lesions which arose were accompanied by a significant viraemia. In turn, extravascular virus deposition occurred in the right knee joint, the precise site of physical trauma 7 years ago. This led to the crippling inflammatory joint response. Our patient's experience is consistent with the experimental fact that minor insults, stresses or tissue damage may serve as a localizing factor for circulating viral particles (Platt, 1969). It is tempting to assume that, had there been no prior joint injury, the viraemia would have remained clinically occult for at no time were his constitutional symptoms significant.

The acute herpetic arthritis observed in our patient duplicates that seen in the classical forms of infectious arthritis due to haematogenous spread of bacteria (gonococcus, meningococcus) or virus (rubella, hepatitis virus) (Ansell, 1978). Similarly, it did not develop into a chronic form, nor have there been any recurrent attacks. The rarity of herpetic arthritis probably reflects the fact that in ordinary attacks of herpes, the viral colonization in the skin is quantitatively too small to induce the degree of viraemia requisite for induction of viral growth at distant sites of 'minoris resistentiae'. Certainly in experimental herpes the size of inoculum determines whether or not central nervous system lesions develop (Wildy, 1967). By contrast, in the highly sensitized patient a small herpetic lesion may produce enough viral particles within the blood stream to trigger erythema multiforme (Shelley, 1967).

In conclusion, arthritis must be considered as a complication of widespread cutaneous herpes simplex, despite its absence in the previously reported cases of disseminated herpes so characteristically seen in wrestlers (Selling & Kilbrick, 1964; Dyke *et al.*, 1965; Porter & Baughman, 1965; Wheeler & Cabaniss, 1965).

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Familial acrogeria (Gottron)

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SUMMARY

A mother and her oldest son suffering from acrogeria are described; in the mother the disease was complicated by elastosis perforans serpiginosa. Microscopic and ultramicroscopic details are given.

Acrogeria is a rare congenital disease described first by Gottron (1941). Its main characteristic is atrophy of the skin of the whole body, but most severe on the hands and feet, resulting in an aged appearance. Occurrence in siblings has been reported by Gottron (1941), but occurrence in two generations has never been described previously.

CASE REPORTS

At examination the mother was 37 years old, the son 12 years. The mother's abnormalities presented at birth, the son's were first noticed when he was 1 year old. Both presented with atrophy of the skin, most marked on the backs of the hands, fingers, feet and toes, extending over the distal parts of the forearms and legs. The atrophy gradually decreased towards the proximal part of the limbs. The veins were showing through and the tendons of the extensor muscles and the bones stood out prominently. The fingers were slender, but broadened at the interphalangeal joints (Fig. 1). The emaciated hands and feet of the mother looked like those of an old woman. A simian crease was present on the palms of both patients. Moreover in the mother there were many fine palmar creases as in Ehlers Danlos syndrome. The mother's fingernails were small, the nails of both big toes showing onychogryphosis. The boy had normal fingernails and small toenails.

The forearms and the legs below the knees (especially of the mother) were remarkably thin, but the muscular strength was normal. In the mother the tip of both thumbs deviated radially.

Both patients had a remarkable face with a thin pinched nose and peculiar wrinkles arranged in semicircles around the corners of the mouth extending to the outside of the eyes (Fig. 2). In the mother the face had an emaciated appearance (Fig. 3), but the boy's face was round. In both, the eyes seemed to protrude but on ophthalmological examination there was no exophthalmos. Their ears were small with absence of the earlobes. The dark blond hair had receded in both cases above the ears and in the temporofrontal area; eyebrows and eyelashes were normal. The palate was arched; the boy's teeth were normal, but the mother's teeth had been removed.

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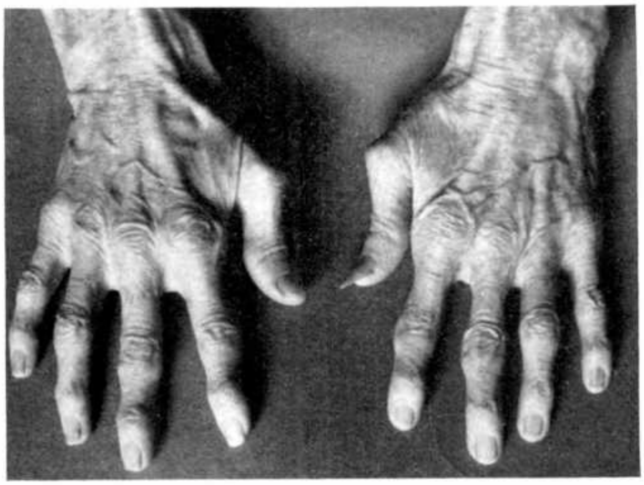

FIGURE 1. Prematurely aged hands of the mother.

The skin of the neck was atrophic with marked wrinkles as in old people. On the trunk blue veins were clearly visible (Fig. 4). Pubic and axillary hair was sparse in the mother, and absent in the boy.

On the extremities and the abdomen of the mother some bruises and many poorly healed scars were present, but there were no extensive ecchymoses or atrophic pigmented scars over the legs and bony prominences. The boy had no bruises at examination but did have a few scars. The mother showed typical lesions of elastosis perforans serpiginosa in both axillae, on the arms, on the neck, the inguinal area and on her thighs. In the axillary region and in the elbow folds the skin had a reticulate pattern. These symptoms were absent in the boy, but on the inner side of his arms and thighs and on the abdomen many fine parallel ridges could be seen. In neither of the patients was the skin hyperextensible. Their joints were not hypermobile.

The mother measured only 157 cm with a body weight of 41·8 kg, the son was 144 cm and 30 kg.

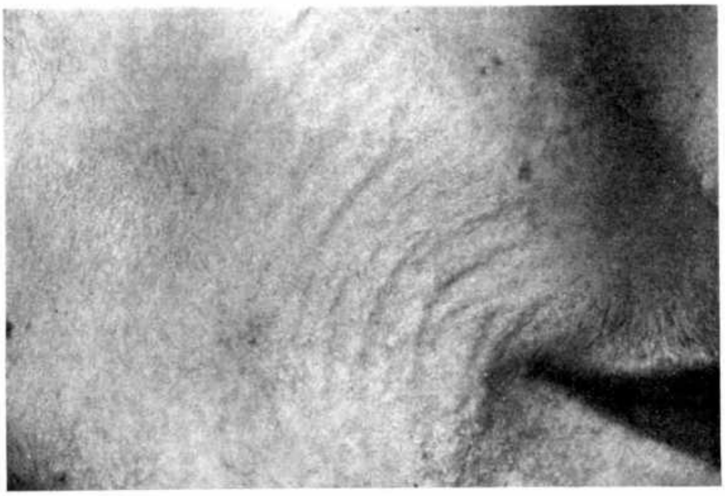

FIGURE 2. Wrinkles around corners of the mouth of the mother.

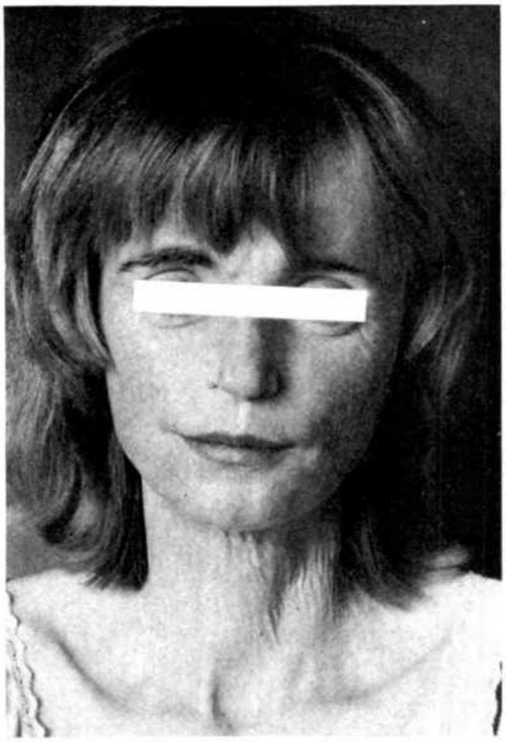

FIGURE 3. Characteristic face and neck of the mother.

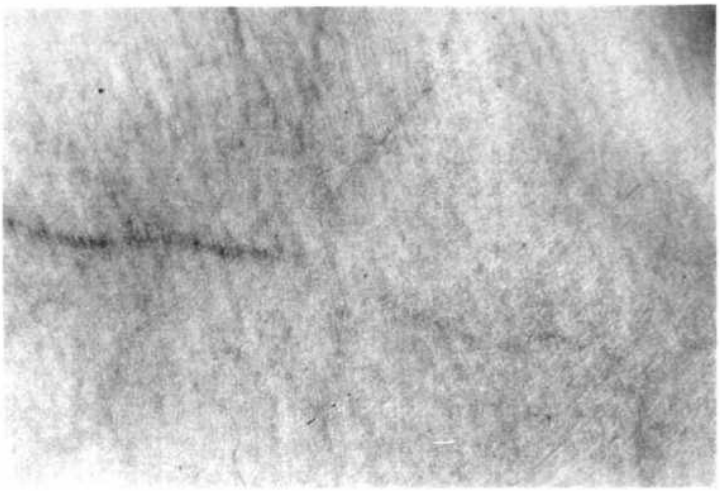

FIGURE 4. Prominent venous pattern on breast of the mother.

INVESTIGATIONS

Histopathology

Light microscopy. Punch biopsies were taken from the dorsum of the hand of both patients (Fig. 5).

The epidermis had for the greater part a normal thickness, but was thin in localized areas; the rete ridges were not flattened. Distinct hypermelanosis was present in the basal layer of the mother's epidermis, but not in the son's. The dermis showed the most conspicuous features. The collagen fibres were swollen, stained poorly and had a 'boiled' appearance. The elastic fibres were disrupted and irregular, in the mother more so than in the son. In both cases several clumps with irregular outlines were present; these clumps stained as elastin (Fig. 6). The vessels especially in the subpapillary layer were markedly dilated with swollen endothelial cells. The sweat glands were lying at a high level in both patients. The subcutaneous fat layer had for the greater part been replaced by connective tissue indistinguishable from the dermal connective tissue. Throughout the dermis and underlying tissue there was some infiltration by lymphocytes, histiocytes and fibroblasts.

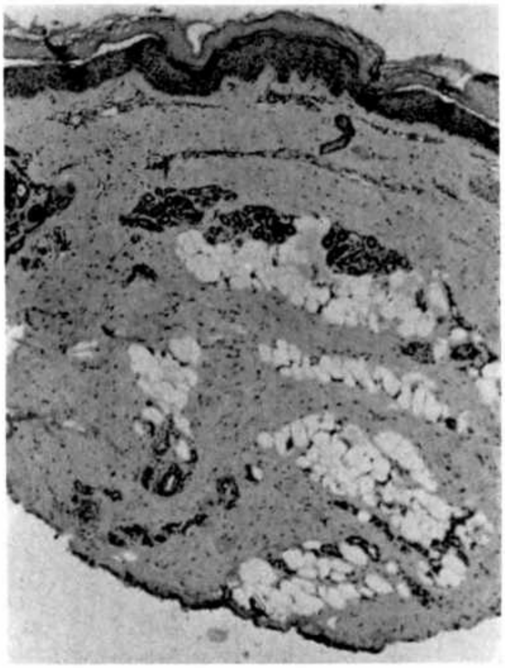

FIGURE 5. Biopsy from dorsal aspect of hand (mother) showing slight hyperkeratosis, sweat glands lying nearer the epidermis than normal, and partial replacement of fat cells by connective tissue.

A biopsy from the abdominal skin of the mother gave a picture similar to that of the hands, but hypermelanosis was present in only a few restricted areas.

The histopathology of the elastosis perforans was typical and needs no special description.

Electron microscopy. Material was obtained from the dorsal aspect of the hand of the mother only (Fig. 7).

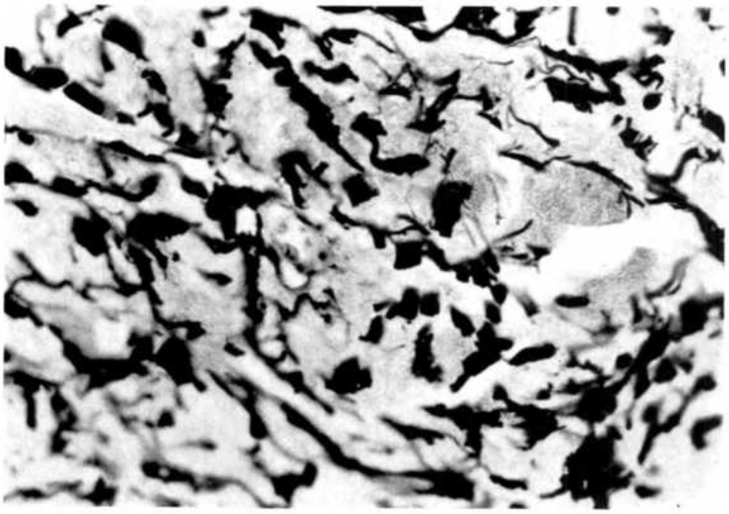

FIGURE 6. Same biopsy as Fig. 5. Verhoeff stain showing irregular and disrupted elastic fibres and formation of several clumps, some with frayed edges.

The pathological changes occurred in the dermis. Most striking was the presence of so-called pseudo-elastin material. It consisted of amorphous masses with frayed edges and with light and dark spots and some granular material. In some of the dark spots fibrillar structures were recognized. There were few normal elastic fibres and some of the collagen fibres were hypoplastic and immature. Intermingled with the pseudo-elastin material and the collagen fibres there was in some places a granular substance. There were large abnormal fibroblasts with many vacuoles and widened endoplasmic reticulum.

Laboratory investigations

Routine laboratory tests were normal in both cases. No clotting defects could be detected. No fungi were isolated from the onychogryphotic nails of the mother.

Radiological findings

In the mother, the skull showed craniostenosis of the brachysphenocephalic type, and hypoplasia of the mandible and the maxillae. Several changes of the bones of hands and feet were present: the terminal phalangeal bones of the hands were hypoplastic; a fusion existed between the trapezial and the trapezoidal bones; the distal phalangeal bones of the third toes on both sides were missing.

In the son, the skull, hands and feet showed no radiological abnormalities.

Ophthalmological examination

Neither patient yielded relevant findings.

Genetics

The mother was the seventh child of a sibship of ten. The other siblings were stated to be normal.

On genealogical investigation no consanguinity was found in five generations between her parents, or between her and her husband. Chromosomal analysis of the mother resulted in a normal female karyotype: 46, XX. No chromosomal abnormality could be detected.

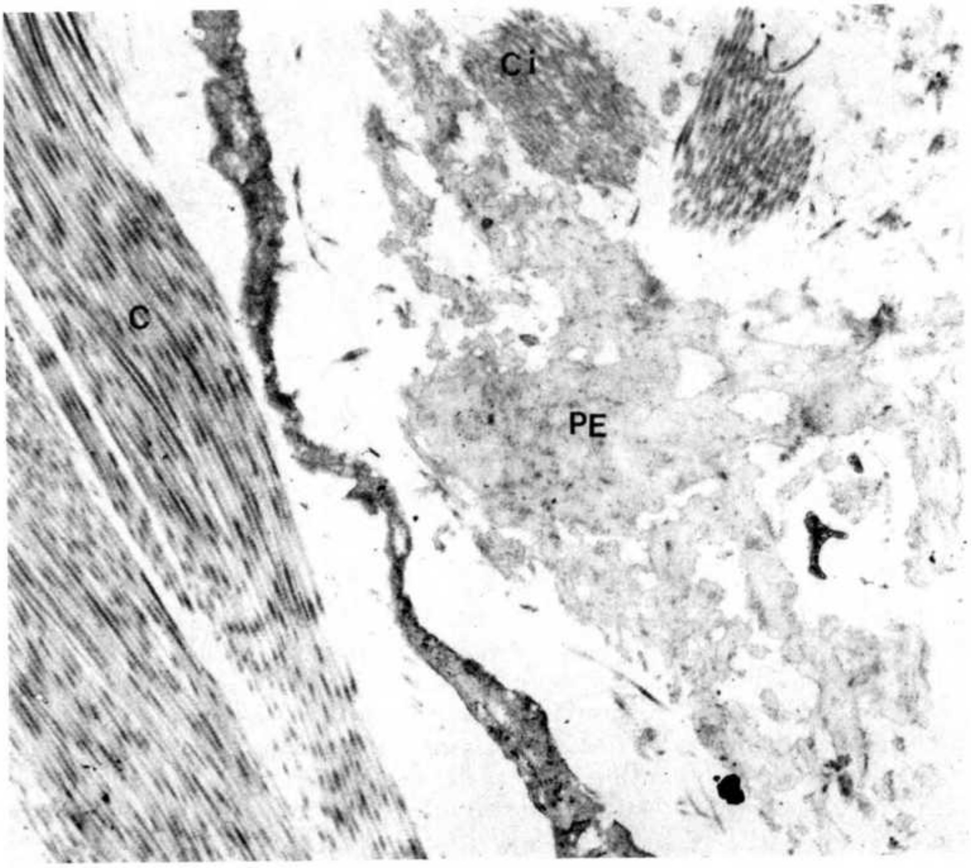

FIGURE 7. Electron microscopy of skin from dorsal aspect of hand (mother) showing papillary layer with pseudo-elastin material (PE) and collagen (C), which is partly hypoplastic and immature (Ci) (× 15,000).

Of the mother's three children only the oldest one was affected, the others being found normal on examination at the age of 10 and 6 years respectively.

DISCUSSION

General

Acrogeria is a rare disease of which we could find only nineteen cases in the literature. Clinical details of these and our own cases are given in Tables 1 and 2.

The preponderance of the female sex is of note; seventeen female and four male patients have been reported. The supposition cannot be rejected that male patients do not seek medical advice as the symptoms cause mainly cosmetic problems. However, this seems insufficient to explain the uneven distribution of the sexes. If the small number of published cases really reflects the sex ratio, the view that this disorder is semi-lethal for the male fetus seems a possible explanation.

Various non-dermatological symptoms have been described of which a small stature and micrognathy are the most frequent; both were present in the mother, but absent in the son.

TABLE 1. Clinical data of patients reported in the literature and in this paper

Reference	Sex, age	Age first noticed (years)	Vessels on trunk	Pigment hands	Elastosis perforans	Nails	Bruises, scars
Gottron (1941)	F, 19	2	–	+	+	Onychogryphosis	
	M, 16	2	–	–		Normal	
Gromzig (1951) Grüneberg (1960)	F, 17	Birth	+	+	+	Onychogryphosis	
Bazex & Dupré (1955)	F, 12	Birth	+	–		Onychogryphosis	+
Calvert (1956) Gilkes, Sharvill & Wells (1974)	F, 6 (22)	Birth	+			Thickened big toe-nails	+
Morris (1957)	F, 7	3					+
Laugier, Gomet & Woringer (1959)	F, 31	6	+	+	+	Koilonychia (fingers) atrophy (toes)	
Lamy *et al.* (1961)	F, 6½	Birth	+	–		Normal	+
Bommer, Kunzer & Hauser (1961)	F, 6	2	+	–		Thin (fingers)	+
Volpato (1961)	F, 7½	6 months		+			
Batschvaroff, Stanischeff & Prikolotina (1961)	F, 23	2				Koilonychia (fingers) atrophy (toes)	
Elefant, Vychytil & Trapl (1965)	F, 3/4	Birth					
Levi *et al.* (1970)	M, 11	3	+			Hippocratic nails	+
Butenandt & Christophers (1970)	M, 13	Birth		+		Thick+onycholysis (toes)	
Raffi, Laurent & Agache (1972) Laurent & Agache (1974) Laurent, Opperman & Agache (1975)	F, 12	Birth	+	+			+
Rosenberg *et al.* (1972)	F, 4	Birth	+			Normal	+
Gilkes *et al.* (1974)	F, 22	Soon after birth	+	–			+
	F, 18	Soon after birth	+				+
Hjortshøj & Heydenreich (1977)	F, 12	4	+			Normal	+
Present cases (1979)	F, 37	Birth	+	–	+	Atrophy (fingers) onychogryphosis (toes)	+
	M, 12	1	+	–	–	Small toes	

TABLE 2. Continued clinical data of patients reported in the literature and in this paper

Reference	Premature birth	Small stature	Micrognathy	Miscellaneous	Familial occurrence
Gottron (1941)		+	+	Well developed panniculus on trunk	+
Gromzig (1951)		+		Pedes Plani	–
Grüneberg (1960)					
Bazex & Dupré (1955)	–	+	–		–
Calvert (1956) Gilkes *et al.* (1974)	+	+			–
Morris (1957)	+				–
Laugier *et al.* (1959)	+		+	Well developed panniculus on trunk	–
Lamy *et al.* (1961)	+	±		Congenital dislocation of hips	–
Bommer *et al.* (1961)	+	–	+	Exophthalmos	–
Volpato (1961)	–				–
Batschvaroff *et al.* (1961)		+	+	Spina bifida occulta	–
Elefant *et al.* (1965)	+			Congenital dislocation of hips	–
Levi *et al.* (1970)	–	–	+		–
Butenandt & Christophers (1970)	+	–		Several fractures	–
Raffi *et al.* (1972) Laurent & Agache (1974) Laurent *et al.* (1975)		–		Pneumoperitoneum, well developed panniculus on trunk	–
Rosenberg *et al.* (1972)	–	+	+	Well developed panniculus on trunk	–
Gilkes *et al.* (1974)	+	+		Eyes appeared prominent, arcus senilis	
		+	+	Raynaud, arcus senilis; both generalized loss of subcutaneous tissue	–
Hjortshøj & Heydenreich (1977)	–	–		Clubfoot	–
Present cases (1980)	+ –	+	+ –		+

Dermatological

The hallmark of acrogeria consists of a striking atrophic appearance of the hands and feet noticed at an early age in all cases, causing an 'old appearance'. Moreover in many cases clearly visible vessels over the whole body and easy bruisability with formation of ugly scars are mentioned. Out of six patients over 19 years old three exhibited elastosis perforans. Various abnormalities of the nails have been seen, including onychogryphosis occurring at an exceptionally early age. Anomalies of the hair as present in our cases have never been published before.

Histology

The histomorphological basis of the clinical symptoms is by no means clear. The descriptions of the histopathology in the literature vary considerably (Table 3). Summarizing the most important histological features from the literature the outstanding pathological changes are present in the dermis, in the collagen as well as in the elastin. Atrophy of the dermis is an almost constant finding

TABLE 3. Histological findings of patients reported in the literature and in this paper

Reference	Site*	Epidermis†	Dermis‡	Subcutis§
Gromzig (1951)	a	1	6, 7, 9, 10, 15, 16	
	c	1	6, 7	
Bazex & Dupré (1955)	a	2, 4, 5	6, 8, 9, 10, 11, 17	18
Laugier *et al.* (1959)	a	1, 4, 5	6, 7, 8, 9, 10	
Grüneberg (1960)	a	2, 5	6, 7, 9, 10, 14, 17	18
Lamy *et al.* (1961)	e	2, 4, 5	6, 14	±19
Volpato (1961)	a	2, 4, 5	8, 9, 14, 17	
	f	1	8, 9	18
Batschvaroff *et al.* (1961)	a	2, 4	6, 8, 9	
	d	1	6, 12	
Levi *et al.* (1970)	d	3, 4	8, 9, 15	
Butenandt & Christophers (1970)	e	2	7, 8, 12	
Raffi *et al.* (1972) }	a	1, 4, 5	6, 7, 13	
Laurent *et al.* 1975) }	a	5	6, 7, 8, 9, 10, 11	18
Rosenberg *et al.* (1972)	g	1	6, 8, 9, 11, 17	19
	b	1		
Hjortshøj & Heydenreich (1977)	e	1	8, 9	18
Present cases (1979)				
1	a	1,5	6, 7, 8, 9, 10, 15, 16	18
	d	1,5	6, 7, 8, 9, 10, 15, 16	18
2	a		6, 7, 8, 9, 10, 15, 16	18

* Site: (a) hand (dorsal); (b) thoracic region; (c) back; (d) abdominal; (e) thigh; (f) leg; (g) foot (dorsal).

† Epidermis: (1) normal thickness; (2) slight atrophy; (3) slight hyperplasia; (4) slight hyperkeratosis; (5) hypermelanosis basal layer.

‡ Dermis: (6) atrophy; (7) sweat glands high in dermis; (8) collagen fibres degenerated, swollen or reduced; (9) elastic fibres fragmented, irregular or thickened; (10) clumps staining as elastin; (11) elastin rare in superficial layer; (12) elastin augmented; (13) elastin normal; (14) vessels normal; (15) vessels widened; (16) cellular infiltration present; (17) cellular infiltration absent.

§ Subcutis: (18) atrophy; (19) normal.

In many cases a variety of changes in the collagen fibres have been observed which are probably all to be regarded as degenerative in nature. Even more striking are the alterations in the elastic fibres and the formation of clumps, staining as elastin. The epidermis shows but minor pathological features: atrophy is slight or absent; marked melanosis is often present in the basal layer, especially in biopsies taken from the hand. Unfortunately the subcutaneous fat has been mentioned in only a few cases. In these cases a normal fat layer has been observed almost as frequently as atrophy.

In our cases also the epidermal changes were of little importance. The outstanding feature was atrophy of the dermis and subcutaneous fat. From the high level of the sweat glands and of lobes of fat cells we concluded in both cases that the dermis was atrophic.

Both collagen and elastic fibres were degenerate, but the elastic fibres were more severely affected in the mother than in the son. Elastin clumps with irregular outlines were present in both cases. The subcutaneous fat had for the greater part been replaced by connective tissue with the same morphological appearance and staining properties as the dermal connective tissue. This caused a false impression of dermal hypertrophy. The replacement of subcutaneous fat by connective tissue has much in common with the so-called proliferative atrophy of fat. It is tempting to conjecture that there has been a subcutaneous fat layer, which by some unknown process has become atrophic and has been replaced by defective connective tissue.

Heredity

In all published cases the parents were reported to be normal and in none was consanguinity known. Occurrence in two siblings has been described only by Gottron (1941) (in accordance either with recessive inheritance or with dominance with low penetrance). The number of siblings has been given in ten cases, but the sex of these siblings is not mentioned in most. In none of the cases has offspring been mentioned, but most patients were too young to have any.

The two cases described by Gottron suggest recessive inheritance, whereas our cases can only be autosomal dominant. The genetic possibilities are either heterogeneity with separate dominant and recessive types or dominance with reduced penetrance. A third possibility could be dominance with high fetal mortality.

Nosology

The nosologic place of acrogeria is disputable especially concerning the relationship to the Ehlers Danlos syndrome. Acrogeria and Type 4 of Ehlers Danlos syndrome have several features in common. Despite the similarity we are of the opinion that these are separate entities. Both syndromes show conspicuous vessels shining through the skin; in both bruisability is present. However, in acrogeria there are neither gross ecchymoses nor intestinal haemorrhages. Wounds heal badly but the extensive scars over the shins and bony prominences or keloids conspicuous in Ehlers Danlos Type IV (Barabas, 1967; Beighton *et al.*, 1969) do not occur in acrogeria. Premature ageing of the hands is a most striking symptom in acrogeria, but has only rarely been mentioned in Ehlers Danlos syndrome Type IV (Pope, Martin & McKusick, 1977).

Minor arguments concern the preponderance of the female sex, the occurrence of onychogryphosis and the presence of elastosis perforans in acrogeria.

We consider acrogeria to be another example of a widespread heritable disorder of connective tissue affecting mainly the cutis and subcutis. The relation to other disorders of connective tissue should be elucidated by biochemical studies.

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Review Article

Tobacco dermatitis

Green or raw tobacco has to be cured, aged and fermented before it is ready to smoke. As is the case with wine, the chemical constituents of mature tobacco depend both qualitatively and quantitatively on a wide variety of factors. The precise chemical composition of a tobacco leaf varies even from one part of the leaf to another (Wolf, 1967). The main variables which determine the chemical content of the leaf as a whole are: the hybrid status of the plant; the soil and climate it grows in; the height of the leaf up the stalk of the plant; the time and method of harvesting; whether it is air-cured or heat-cured (flue-cured); and precisely how it is aged and fermented (Wolf, 1967). As a result a vast range of different waxes, paraffins, fatty acids, organic acids, aldehydes, ketones, phenols, polyphenols, catechols, tannins and other low molecular weight chemicals come to be present in the finished leaf.

It is against this background of chemical variation from one batch of tobacco to another that it is essential to consider the problem of tobacco dermatitis. Convincing reports of allergic contact dermatitis from tobacco are rare among workers in cigarette and cigar factories and excessively rare among tobacco users. Much commoner among cigarette and cigar factory workers handling tobacco are comparatively minor erythemas, urticarias and mild eczemas, associated with the mechanical trauma of operations such as hand stripping and the primary irritancy of tobacco, which is probably due to its content of nicotine and related alkaloids (Knowles, 1913; Rutledge, 1934; Vero & Genovese, 1941; Schwartz, Tulipan & Birmingham, 1957; Gordon-Smith, 1957). Tobacco harvesters, however, can suffer the pallor, vomiting and prostration of nicotine poisoning without any associated dermatitis (Gehlbach *et al.*, 1974).

The earliest reports of tobacco dermatitis are anecdotal only. Blaisdell (1924) described a middle-aged male cigar roller with a work-related hand dermatitis and Davis (1924) a 56-year-old cigar salesman with an episodic hand dermatitis, which spread to his face and trunk. By the time these reports were published the mechanization of cigar making had begun and female cigar making machine operators were replacing male cigar rollers (Samitz, Mori & Long, 1949). A cigar making machine operator, however, still continuously handles the tobacco leaves from which the outer wrapper of each cigar is cut. In general there remains more direct skin contact with tobacco leaf in cigar factories than in cigarette factories, but there have always been certain jobs within cigarette factories where contact could be heavy enough to allow sensitization to take place. Karrenberg (1928) reported a severe dermatitis in a 40-year-old female cigarette factory worker, which started on her hands 3–4 days after she began work on separating tightly packed tobacco leaves. A few days later an acute exudative eczema appeared on her face. The condition cleared when she stopped working and recurred on her return. Patch tests with the tobacco leaves she worked with gave strongly positive, persistent reactions, with vesiculation. The history, clinical findings and patch test results all support a diagnosis of allergic contact dermatitis.

Stauffer (1929) published a report of vesicular hand dermatitis in a 44-year-old woman who had worked in a factory since her youth. Away from the factory her hands healed in 3 months but recurrence occurred repeatedly and increasingly rapidly when she tried to return. A patch test with dry tobacco leaf gave no reaction, but when moist the same tobacco caused severe oedema, erythema and vesiculation. While an aqueous infusion of tobacco in a 1:1000 dilution gave a positive patch test reaction, nicotine in a corresponding dilution did not. Attempts at desensitization by intramuscular injections of tobacco infusion failed.

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Franchi (1937) reported an investigation of hand dermatitis in sixty cigar factory workers. Lesions were most commonly seen on the finger webs, but the palmar aspect of the fingers, the palms and, rarely, the forearms, neck and face were also involved. Standard patch tests with both tobacco and the paste used in the wrapping of cigars were negative. Positive skin reactions were obtained only if a mixture of macerated tobacco and paste, which had been allowed to ferment for 24 h, was applied near the site of hand dermatitis. The latter technique, however, makes it likely that these were false-positive irritant reactions. The epidemiological and clinical findings suggest that, in the majority of cases at least, these workers had irritant rather than allergic contact dermatitis.

Vero & Genovese (1941) described a cluster of three cases of allergic contact dermatitis among cigar makers in the same factory. They put these cases in the perspective of 25 years' experience of hundreds of cases of cigar factory dermatoses, which had all been traced to infections, injuries and contact with paste and other chemicals used in the manufacture of cigars, rather than to hypersensitivity to tobacco. Two of the three patients were middle-aged men who had made cigars for over 30 years and who had suddenly developed work-related hand dermatitis. The third patient was a middle-aged woman who had worked as a cigar maker for nearly 30 years before becoming, for the last few years, a forewoman handling and inspecting tobacco leaves. Her dermatitis was unusual in that it involved her thighs and buttocks as well as her hands; she used to place moist tobacco leaves in her lap while sorting them. In all three cases, patch tests with raw tobacco leaves and with old, dry tobacco leaves gave negative results. Positive patch test reactions in all three cases were obtained only from tobacco leaves which were actually in use in cigar manufacture at the time. In the woman's case the patch test reaction was strong and persisted for several weeks.

As well as false-negative patch test reactions (Stauffer, 1929; Vero & Genovese, 1941), false-positive irritant patch test reactions are readily produced by tobacco. This is illustrated by observations such as those of Swineford & Radford (1948). Of 312 patients with dermatitis patch tested with seventy routine test materials including 'tobacco oil (cigarette and pipe)', twenty-eight reacted weakly and five reacted strongly to tobacco. 'Old pipe tobacco' was tested in one case only and also gave a weak positive reaction. Nicotine salicylate 1% was also among the routine test materials; seventeen out of the 312 patients gave weak reactions to it and two gave strong reactions. The production of weak false-positive irritant patch test reactions with tobacco leaf is discussed by Rycroft *et al.* (1980).

Samitz *et al.* (1949) published an excellent paper on dermatological hazards in the cigar industry, which included an account of hand dermatitis in a large cigar factory over a 9-year period. The majority of cases were seen among female employees who handled and inspected finished cigars coming off cigar making machines. Their work included repairing minor faults on the cigars with gum tragacanth paste. Occasional cases were also seen among female cigar-making machine operators, whose job it was to lay tobacco leaves over an aluminium die for shapes to be cut out from as cigar wrappers. About 80% of these cases were very mild but 1% of cases presented with severe oedematous, vesicular, exudative eruptions on the fingers and sometimes the palms. Only three employees showed positive patch tests to tobacco leaf. One was positive only to Pennsylvania leaf. All three were apparently able to continue their work with impunity, their eruptions appearing sporadically without incapacitating effect. These authors concluded that the vast majority of over a thousand cases of hand eczema seen in the factory over the 9-year period were produced by an irritant combination of wetness of the hands from paste and the alkalinity of tobacco.

The chapter of Schwartz *et al.* (1957) on the tobacco industry summarizes tobacco workers' dermatitis as mainly due to: (1) the mechanical trauma involved in hand stripping; (2) pastes used on cigars and boxes; (3) chemicals used as processing or flavouring agents; and (4) in rare cases, hypersensitivity to tobacco. The authors' own experience included no patch test positive cases of tobacco dermatitis. A number of workers stated that at times they developed a dermatitis beginning on the webs of the fingers and spreading up the forearms, which they associated with handling certain batches of tobacco leaves. Most of these workers were said to handle tobacco before it was fully cured and no such histories were obtained from workers who 'rolled', 'bunched' or sorted finished cigars.

Calnan (1957) reported a case of tobacco dermatitis which was very similar to that of Karrenberg (1928). A few days after a 25-year-old man started work in a tobacco factory, sorting and moving bundles of different varieties of tobacco leaf, he developed an acute erythematous dermatitis of the face, neck, arms, hands and genitalia with oedema, especially of the eyelids. On patch testing he gave a very strong reaction to Turkish tobacco, a strong reaction to American (light) tobacco and a weaker reaction to American (dark) tobacco. In a discussion of the case the medical officer of the patient's tobacco factory commented that, while skin rashes were not uncommon among tobacco workers, this was only the second case of true allergic sensitivity that he had seen.

More recent reports of occupational tobacco dermatitis have appeared from Szegõ & Szilvássy (1966, 1968) and Chanial *et al.* (1970). In Szegõ & Szilvássy's earlier paper they reported the results of patch testing 306 workers in a tobacco fermentation plant and 250 control subjects with various preparations of tobacco leaves, including fresh tobacco. They claimed a 25·8% sensitization rate in their tobacco workers but also found an 11·2% sensitization rate in their control subjects. In addition, 25·4% of patients with various skin lesions showed sensitization. A 5% aqueous extract of fresh tobacco leaves produced most reactions. It is possible that these investigators were, in fact, producing mainly false-positive irritant reactions. In their second paper they reported more convincing patch test results. This was a study of 268 female workers handling cured tobacco leaves. There was no dermatitis among fifty-seven permanent workers and only nineteen cases among 211 seasonal and new workers. Only in five of these women did their rashes not disappear spontaneously. Four of these five women were found to give positive reactions to 5% aqueous extracts of tobacco; all other women had negative patch tests. These results appeared to confirm that workers with self-limiting rashes were not exhibiting desensitization, but merely that they had never been sensitized in the first place.

Chanial *et al.* (1970) reported nine tobacco workers with hand dermatitis, in one of whom the entire face was also involved. Scratch tests were done with dry tobacco and with tobacco moistened with sweat. They were positive in three cases, doubtful in two and negative in four. Changes of job within the factory apparently solved the problem in all cases. This report is difficult to assess in view of the use of scratch tests rather than patch tests. The worker with facial involvement is reminiscent, however, of the genuine cases reported previously by Karrenberg (1928) and Calnan (1957).

Although chemical additives in tobacco have been much cited in the literature as causes of dermatitis (Schwartz *et al.*, 1957) reported cases of allergic contact dermatitis from this source are extremely few. Newman (1938) found that a cigarette smoker, with dermatitis on the fingers in which he held his cigarette and on his upper lip, was allergic to diethylene glycol. This was present in a brand of cigarette that he had recently started smoking. Unna & Schulz (1963) reported allergic contact dermatitis from triacetin, an additive in the filter of cigarettes, in a 24-year-old cigarette factory worker.

Allergic contact dermatitis from tobacco among consumers must be extremely rare. Cormia & de Gara (1965) claimed to have reported the first case of contact allergy to tobacco in a 35-year-old man with intermittent vesicular hand eczema. Their case report is unconvincing clinically, however, and intradermal tests were used, rather than patch tests, which gave immediate rather than delayed reactions. Weary & Wood (1969) claimed to have reported the first case of contact allergy to tobacco smoke residues in a 20-year-old male. This report is more convincing. Shanon & Tas (1958) described a 72-year-old man with dermatitis around the nostrils who gave an eczematous patch test reaction to his snuff tobacco. However, six out of ninety-seven control subjects with eczema also gave eczematous reactions to the same tobacco and snuff tobacco often contains additives. Respiratory tobacco smoke allergy has been a subject of controversy amongst allergists for many years. A recent paper by

McDougall & Gleich (1976) concluded that there was no convincing radioallergosorbent test (RAST) or skin test evidence for it. Allergic rhinitis and asthma from tobacco leaf among tobacco workers has been better substantiated (Gleich *et al.*, 1980). These and many other conditions which have been attributed to tobacco allergy are discussed in a comprehensive review by Silvette, Larson & Haag (1957).

The precise allergen or allergens in cured tobacco have not yet been identified, but nicotine itself is not the sensitizer (Silvette *et al.*, 1957). Mitchell & Rook (1979) have also recently reviewed the dermatological effects of tobacco. The method of investigating patients with suspected tobacco dermatitis is discussed in a paper to which reference has already been made (Rycroft *et al.*, 1980).

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Book Reviews

Textbook of Dermatology. Edited by Arthur Rook, D.S.Wilkinson and F.J.G.Ebling (1979) 3rd Edition. Oxford: Blackwell Scientific Publications, 3rd edn, Pp.2550, 1130 illustrations. Two volumes, Price £87.50.

The hefty two volume bulk of this much respected tome sets a daunting task for the reviewer. It is choice but expensive meat, working out at about £6.15 per lb., but it keeps for ever. It is as usual beautifully produced (although the print seems marginally less legible this time), a work which moth and rust will not easily corrupt, though thieves might wish to break through and steal. The blue volumes of the first edition and the green of the second now stand beside the red of the third in our small departmental library. The former, although somewhat worn and greasy with constant use, are serviceable and intact, in contrast to the single volume of an excellent and in some respects complementary transatlantic text, whose back is broken and whose pages have begun to exfoliate.

Keeping up to date has been a major concern of the editors, and considering the size of the task confronting them they have done well. Some new sections have been added; sometimes large pieces have been rewritten or revised. Always care has been taken to quote recent references, so much so that the result almost has a static quality. The lady stares at you from the canvas fixedly, painted in the fashion of the late seventies, but if you want to know her story you will have to seek elsewhere. Such an absence of historical perspective is disturbing in a scholarly book. Yet on page one Arthur Rook says: 'The frontiers of each disease are constantly shifting and a true understanding of their present significance is impossible without knowledge of their historical development.' The reason for this omission is said to be lack of space, but there is much repetition in the course of 2366 pages, and not all the contributors are as succinct as Peter Samman. There is a temptation for authors to be verbose when confronted by impenetrable dermatological mysteries, just as dermatologists' letters tend to be longest when their confidence is least. Much of the repetition arises from dealing with a subject first under its disease, and later in a regional essay. The book would gain in character and usefulness if it confined itself chiefly to a disease-by-disease presentation in which the contributions of the clinician, the scientist the histopathologist and the dermatological historian were melded into a whole. One great advantage of the clinician, one basis of his authority, is the opportunity he gets of observing events unfolding. This is a clinician's book *par excellence*, and he deserves to be given a clinician's perspective. It would also be nice to have an explanation of some of those strange names by which dermatologists know skin diseases, as Sir Norman Walker did in his textbook. What, for example, does *diutinum* mean?

Having said this I must record my continued admiration for this book, admiration that borders on veneration. It is my standard text, my first port of call in every clinical problem. The insertion of references at frequent intervals is a specially helpful feature (even if I beg leave to wonder whether an occasional author has read, marked, learned and inwardly digested some of the references he has quoted). The only coloured picture—of tinea capitis seen clinically and under Wood's light—makes its mark, and pleads mutely for some friends to comfort its loneliness. Most of the black and white illustrations are good, some outstandingly so; for example those of syphilis, and in the magnificent chapter on the mouth (a regional study that is fully justified because it is so specialized and so little known). Chapters that appealed to me specially were those on immunology, on lupus erythematosus, on contact dermatitis and on photosensitivity. For the confused and hard worked clinician the chapter on atopic dermatitis is a pearl. Talking of dermatitis I still find that the nettle of eczema/dermatitis has been grasped rather feebly, a point made when reviewing the first edition for the *British Medical Journal*. The time has come for us to call it dermatitis or eczema, and most people seem to favour dermatitis. When that day dawns what will happen to that bastard term 'infective eczematoid dermatitis' poor thing? I shall not weep for its demise.

Weeks spent dipping at random, followed by three solid days for my final run left me dizzy, mentally constipated, overawed and battered by syndromes; but this is a book to refer to, not to read in large chunks. The initial state of shock induced by this orgy and the sense of my own ignorance that it inspired, gradually passed off and allowed me to write thus far. I should like to spend the rest of my review in particularizing upon certain items that caught my eye.

Leprosy is a difficult enough subject, but what does figure 23.1 mean? Another figure I found very difficult was 9.2 which aimed to show four possible effects of vascular damage. Psoriasis is often itchy; so is pityriasis rosea, though the text does not convey this adequately. Pityriasis rosea may be a virus disease, but I doubt whether the evidence justifies classing it as one yet. Is excessive normal keratin scaly? The early diagnosis of tuberous sclerosis could have been mentioned under the uses of Wood's light on page 54, and the Ferguson Smith self-healing epithelioma (apologies to Messrs Sanderson and Mackay for using that despised surgical term 'epithelioma') could have been included in the list of autosomal dominant diseases on page 103.

Is the Sulzberger-Garbe syndrome really confined to Jews? Surely the concept of sweat retention as a cause of pompholyx has been abandoned, and so the term 'dyshidrosis' should go with it. The great variety of triggers initiating lichen simplex is not described, nor is that kind of lichen simplex affecting the palms and soles, that responds well to superficial X-ray therapy.

Who was the author who recorded the spreading of leprosy by tattooing? Mosaic warts, so difficult a problem in the clinic, and so different from the usual painful plantar warts, are barely mentioned. Surely liquid nitrogen is the treatment of choice for hand warts in a hospital clinic, rather than carbon dioxide snow. Why continue the term 'Kaposi's varicelliform eruption'? Is it really true that it is impossible to distinguish clinically between herpetic and vaccinial cases? In my experience involvement of the Public Health authorities in the management of papular urticaria creates more problems than it solves.

Are animals civilised by cold? It is a stimulating thought. Perhaps many psoriatics take to the bottle to escape their dreadful disease. A section on the adverse and disruptive social implications of psoriasis might have a civilising influence on those dermatologists who choose to ignore this cold fact of life. It is not true in Glasgow to say that the topical application of corticosteroids is central to the out-patient management of psoriasis. The section on methotrexate is excellent; this treatment, for all its drawbacks, being worth serious consideration in socially crippling psoriasis.

Isn't it surprising that rosacea responds to oral tetracyclines; exciting, interesting, provocative, stimulating?—apparently not. I was disappointed at the half-hearted condemnation of the topical use of antihistamines. Brain damage is not mentioned as an accompaniment of X-ray epilation of the scalp hair. I could find no reference to polished and bevelled finger nails in pruritus, to Blaschko's lines, cutaneous foci of haemopoiesis in the infant, emotional tension as a factor in nummular dermatitis or in acne necrotica, the tendency of hands and feet to react in sympathy with one another, the cold water treatment of burns and scalds, the occurrence of lupus vulgaris on the buttocks, the indurated type of scurvy, the remarkable aggravation of eczema induced by eating nuts in some cases of Netherton's syndrome, and that anhidrosis can be a cause of pruritus.

The term 'Toxic epidermal necrolysis' should be replaced by 'the scalded skin syndrome', so that the staphylococcal scalded skin syndrome (not named in the paediatric section) would fall into place naturally. Staphylococcal epidermolytic toxin, which is the key to understanding the effects of dermopathic strains of *Staph. aureus*, was mentioned in only one of three possible places (by Sneddon), and did not reach the index.

I make no apology for having written a long review. This is a long and important book, a worthy successor to the two earlier editions. It is the best text on clinical dermatology in the English language. But it could still be improved, and it will be if readers will correspond with contributors to this end, a form of criticism that complements the necessarily limited viewpoints of any individual reviewer.

ALAN LYELL

Experimental Skin Grafts and Transplantation Immunity; A Recapitulation. D.L. BALLANTYNE and J.M.CONVERSE (1979) Springer-Verlag, New York; Heidelberg; Berlin. Pp. 192. 60 illustrations. Price US $33.60; D.M.60.

This is an excellent small book written by two persons who themselves have greatly contributed to the history of transplantation immunity which they review in depth. The seven chapters cover vascularization of skin grafts, autografts and allografts, acute and chronic rejection patterns of first-set skin allografts and survival and rejection of second-set skin allografts. Reaction patterns and dosage phenomenon of skin xenografts are also described. The illustrations are well selected and some 400 references provided. The morphology, histochemistry and biochemistry of grafts have been extensively examined, using very varied techniques, and the information so obtained is of value to anyone interested in the behaviour of epithelium and its blood supply.

The authors have indicated where there is work still to be done and this reviewer is surprised by how little can be concluded from what is known so far. It is clear what happens, but why it happens has not yet been assessed.

This monograph is a generous provider of facts upon which to base informed guesses. The issues raised are not confined to transplantation and immunity. It is a well written enjoyable and mysterious tale.

T.RYAN

Diagnosis of Skin Disease. GERALD S.LAZARUS, and LOWELL A.GOLDSMITH with the assistance of MICHAEL D.THARP (1979) Philadelphia: F.A.Davis. Pp. 506, illustrations, many in colour. Price £3.25.

The detailed analysis of the morphology of skin lesions has in some countries fallen into disrepute in the last twenty or thirty years. It did so because some schools of dermatology placed too much emphasis on minor, and sometimes irrelevant, variations in morphology as a basis for elaborate classifications and cumbersome nomenclature. Recently several authors have strongly deplored this neglect of morphology, since the careful and critical study of cutaneous physical signs in relation to the physiopathological mechanisms which give rise to them can provide valuable, sometimes indispensable, clues to the correct diagnosis.

This new book from the Division of Dermatology, Duke University Medical Center, considers every form of skin lesion—erythema, papules, nodules, and so forth—and further sub-divides each according to other morphological criteria, e.g. papules, brown, single or multiple. For each disease thus classified an outline diagram indicates the distribution of the lesions and one or more photographs illustrate their usual appearance. Selected lesions are illustrated also in colour. The book ends with tables of differential diagnoses.

In their preface the two senior authors state that they 'aim to present a clear approach to dermatological disease that will enable any practising physician to arrive at a reasonable differential diagnosis through a logical approach'. They have succeeded in their aim. The book has been so carefully planned and includes so many of the rarer diseases that there can be few dermatologists who will not find it useful. Most of the illustrations are good and some are outstanding but a few are at best uninformative and should be replaced in the future editions which will certainly be called for.

This is a practical book for regular consultation in the clinic or consulting room and the binding should have been stout, durable, waterproof and dirt-resistant. The Publishers have unfortunately chosen to cover the book in absorbent white cloth which marks easily and the binding, of this reviewer's copy at least, is far from robust. Perhaps it may be possible for future printings to be better served by the binders.

ARTHUR ROOK

Behçet's Syndrome. Clinical and Immunological Features. Proceedings of a Conference Sponsored by the Royal Society of Medicine, February 1979. Edited by T.LEHNER and C.G. BARNES (1979) London: Academic Press. Price £16.40.

This is the second monograph on Behçet's Syndrome to be reviewed in these pages within months. Like *Behçet's disease*, Proceedings of an International Symposium (Istanbul 1979, Excerpta Medica Amsterdam—Editors: Dilsen, Konice, and Ovul) it is the printed proceedings of a Conference, this time at the Royal Society of Medicine, and it took place merely a year ago. A glance at the separate chapter that lists all the references, an admirable device to eliminate repetition, will reveal numerous up-to-date quotations, although disappointingly the titles of these papers are not presented.

The book begins with a flourish, as if to deny its own eponymous title, by giving credit for their descriptions of the disease to several observers before Behçet, the best known of whom is a physician by name of Hippocrates, whose contribution is translated for the benefit of non-humanists into quaint vernacular. The three main sections separate ten chapters on immunology from eleven on clinical aspects, and three on treatment. Some of the chapters might be a little over-chatty and others a little over-burdened with detailed case histories, matters which remind one of the book's lineage and origins in the lecture theatre. One will look in vain for any deep comment on pathology or on mechanisms that seem to be prominent in producing physical signs as, for example, thrombosis.

The skin receives many mentions throughout, but apparently was not considered a suitable subject to be accorded a chapter on its own, despite the editor's own classification of the disease into four types; muco-cutaneous, arthritic (two chapters); neurological (one); and ocular (three). The editors have succeeded in presenting a useful compendium concerning clinical and immunological aspects of the disease, and one of them, Professor Lehner, proves himself also to be most energetic, for no less than 16 of the 24 chapters bear his name, a feat when 33 contributors are listed withal.

This volume and the other one reviewed earlier will provide a sound basis for anyone planning to present, study, or write up a patient with Behçet's disease and will enable him to pinpoint how and where he can contribute new knowledge, which could prove a blessing also for future followers in this much muddied field.

P.W.M.COPEMAN

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News and Notices

European Society for Comparative Skin Biology

A one day symposium on 'The Glands of the Skin' has been arranged by the Skin Club and the European Society for Comparative Skin Biology, to be held at the Institute of Urology, London, on Thursday, October 30th, 1980. Details may be obtained from Dr R.I.C.Spearman, Department of Dermatology, School of Medicine, University College London, University Street, London WC1 E6JJ.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents.*

Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations,* A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'Manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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British Journal of Dermatology

The British Journal of Dermatology is owned by and is the official organ of the British Association of Dermatologists. It is also the official organ of the Nederlandse Vereniging voor Dermatologie en Venereologie (Netherlands Association for Dermatology and Venereology).

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REFERENCE:
British Medical Journal, 1978, **2**, 84.

PL 0173/0030

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Dosage:
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Contraindications, Warnings etc:
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in acne

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is a significant improvement over ordinary tetracyclines

HIGH PENETRATION[1,2] EVEN INTO SEBUM.

HIGH ACTIVITY
EVEN IN TETRACYCLINE-RECALCITRANT ACNE.[2]

SIMPLE DOSAGE
B.D. DOSAGE CAN BE TAKEN WITH MEALS.

LOW INCIDENCE OF SIDE EFFECTS[3]

1. Cullen, S.I., Cutis, 1978, **21**, 101
2. Becker, F.T., Cutis, 1974, **14**, (4), 610
3. Cullen, S.I. and Cohan, R.H., Cutis, 1976, **17**, 1208

NEW LOW DOSE

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Dosage Adults and children over 12 years. One tablet twice daily. Minocin may be taken with meals. Treatment of acne should be continued for a minimum of six weeks. **Contra-indicati**
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and side effects Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discolouration of teeth is a possible sequela of treatment during pregnancy
early childhood (up to 8 years of age). **Presentation** Beige tablets containing 50mg of minocycline as the hydrochloride, supplied in bottles of 100 at a basic NHS cost of £18.00. PL/0095/(
Full prescribing information is available on request. MINOCIN is a trademark.

Lederle Laboratories Division of Cyanamid of Great Britain Ltd, Fareham Road, Gosport, Hants PO13 0AS (Tel: 0329-236131).

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And Alphosyl HC is preferable on safety grounds since it does not contain a fluorinated steroid.

Alphosyl HC

hydrocortisone 0.5% with allantoin and coal tar extract.

purpose-designed for psoriasis

*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol 33 No. 4, 106.

resentation A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone, s.p. 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogenous and is light tan in colour. **ses** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage nd Administration** Apply two to four times daily massaging vigorously into the affected areas. **ontra-indications and Warnings, etc.** In infants long term continuous therapy should be avoided. Adrenal

can cause abnormality of foetal development. The relevance of this finding to human beings has not be established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amount for long periods. **Pharmaceutical Precautions** Store at room temperature. **Package Quantities** Tub 30gm and 45gm. **Basic NHS cost** £1.78 for 30gm, £2.52 for 45gm. **Product Licence No.** 0036/0026. Further information is available from

No need now to sacrifice efficacy for safety because Eumovate fulfils the need for a corticosteroid preparation with greater topical activity than hydrocortisone, yet with a wide margin of safety.

Eumovate

(clobetasone butyrate)

a unique balance of efficacy and safety

Prescribing information

Uses

Eumovate is suitable for treating the mil forms of eczema, seborrhoeic dermatit and other steroid responsive skin conditions.

Dosage and administration

Apply up to four times a day until improvement occurs, when the frequen of application may be reduced.

Contra-indications

Bacterial, fungal or viral diseases of the skin.

Precautions

In infants and children, long-term continuous topical corticosteroid therap should be avoided where possible, as adrenal suppression can occur. In infan the napkin may act as an occlusive dressing, and increase absorption.

Appropriate antimicrobial therapy shoul be used concurrently whenever treating inflammatory lesions which have becom infected.

Any spread of infection requires withdrawal of topical corticosteroid therapy and; possibly, commencement systemic chemotherapy.

As with all corticosteroids, prolonged application to the face is undesirable. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately. If large areas were to be treated, some patients could absorb sufficient steroid to cause transie adrenal depression despite the low degr of systemic activity associated with clobetasone butyrate.

Local atrophic changes could occur whe moisture increases absorption of steroid but only after prolonged use.

Basic NHS cost (exclusive of VAT) 25 gram tube £1·63 (also available in 100 gram tubes).

Product Licence numbers

Cream: 4/0233
Ointment: 4/0254

Glaxo

leaders in topical steroid thera

Further information on Eumovate (trade mark) is available from:
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rescribing Information: Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white pow
ntaining 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections. **Dosage and Administration** *Crea*
tomiser Spray Apply to the affected area 2 - 3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylac
ainst re-infection. **Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near a
ked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled. **Product Licence Numbers** Cream: PL 0010/0016
tomiser Spray: PL 0010/0060 Powder: PL 0010/0067 **Basic NHS Prices** Cream: 20g, £1.52 50g, £3.55 Atomiser Spray: 40ml, £4.08 Powder: 30g, £1.10. Further information
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Why mess about with topical antifungals when Grisovin gets right to the root of the problem?

GRISOVIN

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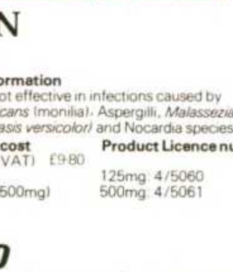

THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily. *Children:* usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease.

Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost		**Product Licence numbers**
(exclusive of VAT)	£9·80	
Bottle of 100 tablets (500mg)		125mg: 4/5060 500mg: 4/5061

Glaxo

Full prescribing information on Grisovin (trade mark) is available from:
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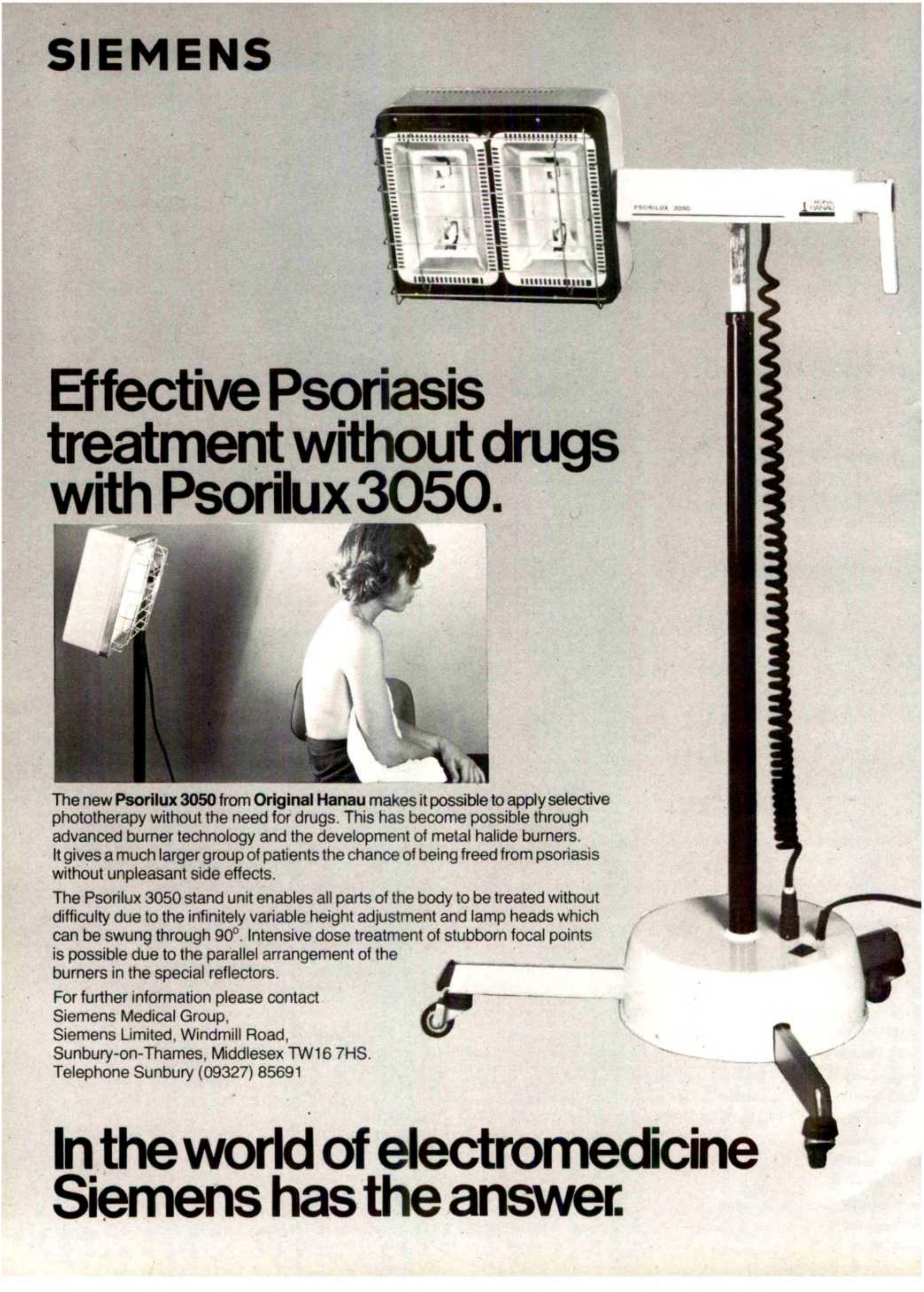

SIEMENS

Effective Psoriasis treatment without drugs with Psorilux 3050.

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For further information please contact
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✱Low Sensitization[3]

✱Highly acceptable[4]

Available as ointment or cream.

Locoid C

Hydrocortisone 17 butyrate 0.1% chlorquinaldol 3.0%

When infection is present or suspected.

References:

(1) Amer Pract. & Digest of Treat. (1955) 6 8
(2) Transact New York Acad of Sci. (1955) 10 161
(3) Arch. Derm (1972) 106 335
(4) Rocky Mtn. Med. J. (1955) 52 6

Prescribing Notes

Dosage & Use. Apply to the affected part two to four times a day, until the infection is resolved. If the underlying condition persists treatment may be continued for up to 14 days and no longer. No more than 60g may be applied during this period. Do not use under occlusion in the presence of infection.

Contra Indications. As with all topical steroids, Locoid C preparations are contraindicated in the presence of viral, tubercular or syphilitic lesions.

Other Precautions. Avoid contact with the eyes. Not to be used in pregnancy in large amounts or for long periods.

In infants, avoid long term continuous therapy; especially avoid occlusion.

Cost Basic NHS price £2.39 for 30g

P/L nos Locoid C cream 0166/0056
Locoid C ointment 0166/0057

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Prescribing information

Dosage and administration
Apply sparingly once or twice daily until improvement occurs. Treatment should not be continued for more than four weeks without review.

Side effects
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism.

This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing

Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications
Rosacea, acne and peri-oral dermatitis. Bacterial, fungal or viral diseases of the skin.

Basic NHS cost (exclusive of VAT)
Dermovate Cream or Ointment 25 gram tubes £2·07 (also available in 100 gram tubes).

Presentation	**Product Licence number**
Dermovate Cream	4/0219
Ointment	4/0220

Glaxo
Leaders in topical steroid therapy

Further information on Dermovate (trade mark) is available from:
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Another Munksgaard Journal

Allergy

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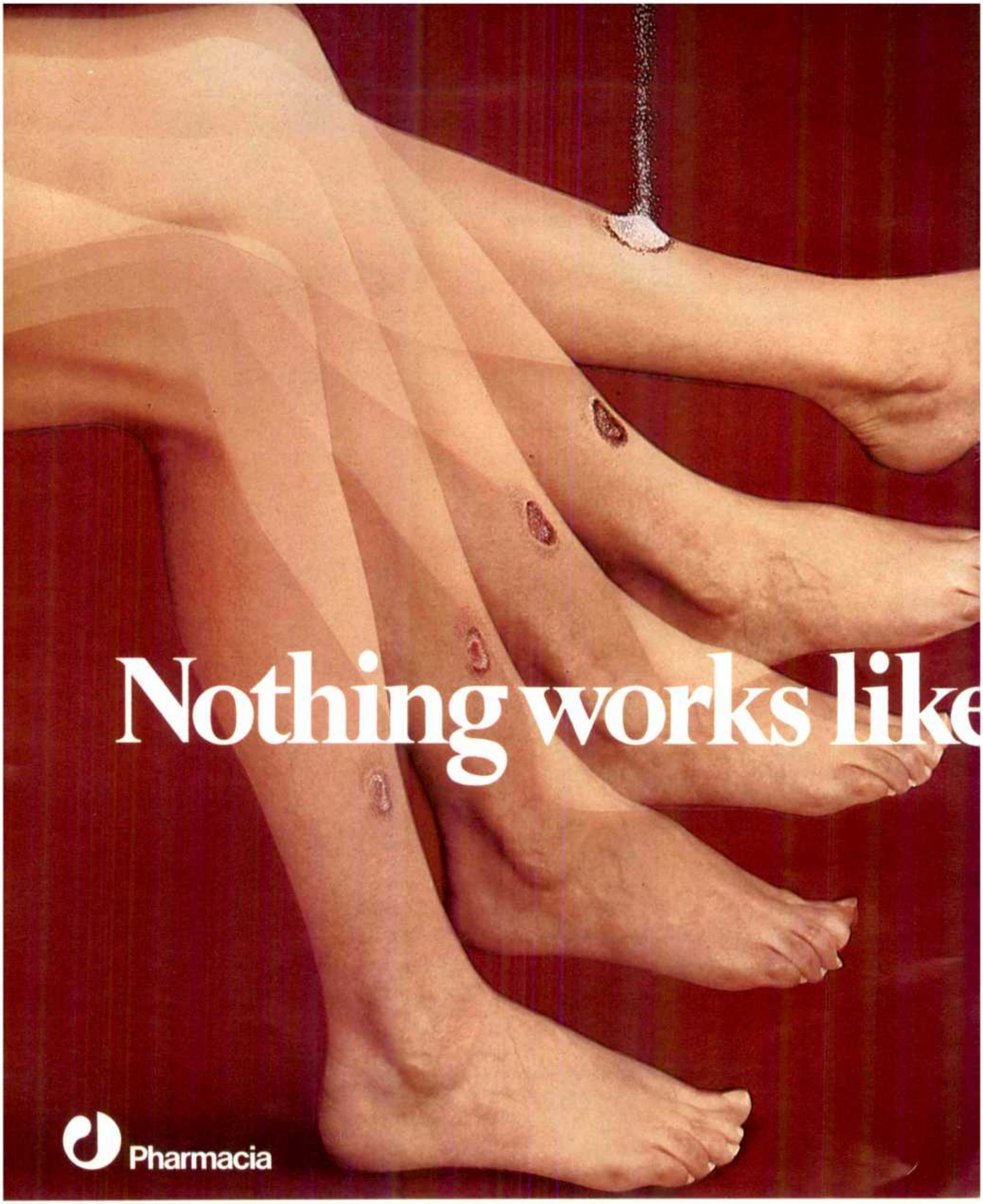

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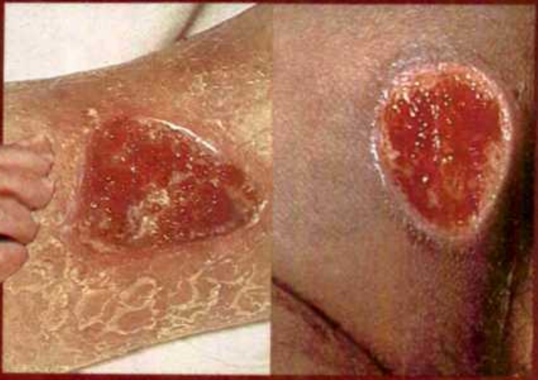

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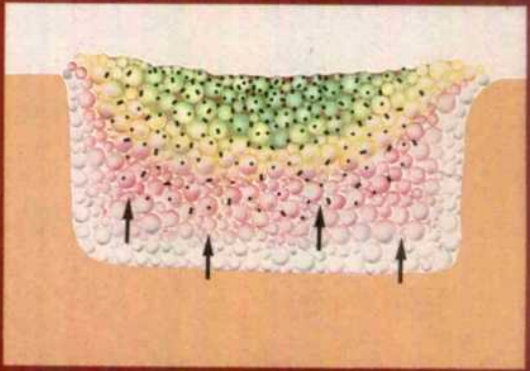

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[1] Thorne N. (1979) Brit. J. clin. Pract. **33**, 21

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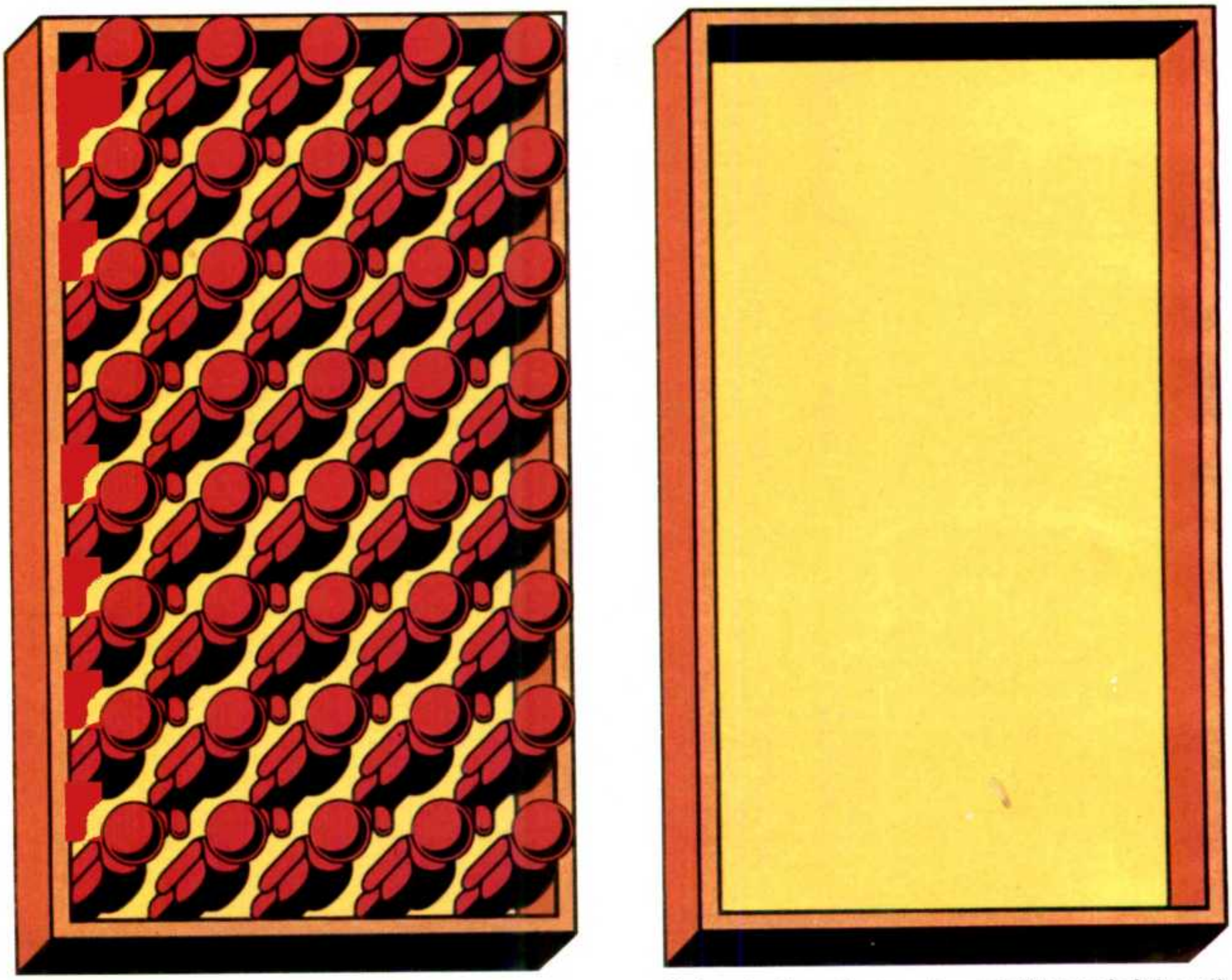

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1. *British Journal of Dermatology*, 1979, **100**, 475

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Clinical and Laboratory Investigations

Pharmacological responsiveness of the myoepithelium of the isolated human axillary apocrine sweat gland

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SUMMARY

Human axillary apocrine glands are endowed with well developed myoepithelium, but its pharmacological responsiveness has remained unknown. The present study is part of our efforts in determining the role(s) played by exocrine myoepithelium in the overall secretory processes. Single human apocrine glands were isolated from biopsy skin specimens and were stimulated with various pharmacological agents *in vitro*. Tubular contraction was recorded photomicrographically and was interpreted as being due to myoepithelial contraction. The tubular contraction was induced by stimulation with phenylephrine (5×10^{-6}M) or adrenaline (5×10^{-6}M) but not with isoproterenol (5×10^{-6}M) or acetylcholine (5×10^{-6}M). A higher concentration of acetylcholine (10^{-4}M) produced a minor degree of contraction. The phenylephrine-induced contraction was blocked by phentolamine (10^{-5}M) or by EGTA (5 mM) but not by propranolol (10^{-5}M) or by atropine (10^{-5}M). It was concluded that the contractile response of the apocrine myoepithelium is selectively controlled by the alpha-adrenergic stimulation. The possibility is discussed that the major function of the apocrine myoepithelium is not just the pumping out of the preformed sweat but is, as in the eccrine sweat gland myoepithelium, the provision of structural support for the apocrine secretory epithelium to withstand the increase, if any, in the luminal hydrostatic pressure during apocrine sweat secretion.

Myoepithelium is ubiquitously present in many exocrine glands such as eccrine sweat glands, apocrine glands, lacrimal glands and salivary glands (Ellis, 1965). The role of the myoepithelial cells in the secretory process has remained unknown; however, the expulsion of the preformed secretory fluid by myoepithelial contraction has been the most widely accepted hypothesis (Hurley & Shelley, 1954; Nicolaides & Sivadjian, 1972).

The myoepithelial cell lines the outermost layer of the secretory tubule just inside the basal lamina, is spindle-shaped and is filled with irregularly arranged bundles of myofilaments. It has many morphological (Ellis, 1965) and functional (Sato, Nishiyama & Kobayashi, 1979) features similar to, if not identical with, smooth muscle cells. Previous studies from the author's laboratory (Sato, 1977;

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Sato *et al.*, 1979) have demonstrated that in the eccrine sweat gland: (1) myoepithelial cells do contract in response to cholinergic stimuli but not to other stimulants of sweat secretion such as alpha- or beta-adrenergic agents, a calcium ionophore A23187, prostaglandin E_1 etc.; (2) there is no pulsatile contraction during continued exposure to acetylcholine; (3) there is minimal preformed sweat in the lumen of the secretory tubule so that the myoepithelial contraction does not push out any appreciable amount of sweat *in vitro*; (4) there appears to be a quantitative agreement between the maximal contractile force (i.e. 20 mg force per tubule) and the maximal mechanical tolerance of the tubular wall as determined by the maximal stopped flow luminal hydrostatic pressure (Schulz, 1969). These observations have led us to propose that the primary function of eccrine myoepithelium is the provision of structural support for the tubular wall during secretion of sweat (Sato *et al.*, 1979).

The apocrine sweat gland is also endowed with well developed myoepithelium. However, the functional aspects of the apocrine myoepithelium have not been well studied. The apocrine gland offers a unique opportunity for studying the myoepithelial function, because in contrast to the eccrine sweat gland the apocrine secretory tubule has a large luminal space and retains a large amount of preformed sweat in the lumen. Thus it is possible to analyse the extent to which the apocrine myoepithelium-induced 'pumping-out' of preformed sweat, if at all, might contribute to the overall apocrine secretory activity. In the present study we have used photomicrographic methods to show that the apocrine myoepithelium contracts in response to alpha-adrenergic stimulation but not to cholinergic or beta-adrenergic stimulation. This observation, coupled with our preliminary finding that alpha-adrenergic stimulation is the least effective stimulant of apocrine sweat secretion (Sato & Sato, 1979), will be discussed in terms of its possible significance in the overall apocrine secretory process.

MATERIALS AND METHODS

Elliptical surgical biopsy skin specimens, one cm long and 0·5 cm wide, were obtained from the axillae of five Oriental and three Caucasian female volunteers, 16–22 years of age, under local anaesthesia using 1% lidocaine or 1% lidocaine with adrenaline at a concentration of 1:100,000. Since no significant difference was noted in the myoepithelial response to drugs between the two races and between the two local anaesthetics the data are reported together. The tissue was sliced and washed in several changes of ice-cold (4°C) modified Krebs bicarbonate Ringer's solution (KRB) containing 125 mM NaCl, 5 mM KCl, 1·2 mM $MgCl_2$, 1·0 mM $CaCl_2$, 25 mM $NaHCO_3$, 1·2 mM NaH_2PO_4, 5 mM glucose, and 50 mg per 100 ml fatty acid-free bovine serum albumin (Sigma). Single apocrine glands were isolated under a stereomicroscope by gently teasing away the fatty tissue and collagen fibres around the apocrine gland with two pairs of Dumond No. 5 tweezers (Ladd Research Burlington, VT) in a dissection chamber (Sato, 1973) kept at 10°C.

In Fig. 1, two isolated human axillary apocrine glands are shown still attached to a hair follicle. The ductal portion of the apocrine gland is usually short and has a smaller diameter than the secretory portion. Thus the secretory portion was easily identified under a microscope. The isolated gland was transferred to a thermostatically controlled (37°C) microchamber placed on the mechanical stage of a microscope. The chamber consisted of a narrow groove, 100 μm in depth, the top of which was covered with a cover glass. Warmed KRB was continuously infused from one open end of the slot and the overflow was removed from the other end by continuous suction. Pharmacological agents tested include acetylcholine HCl, L-Phenylephrine HCl, L-adrenaline bitartrate, DL-isoproterenol sulphate, atropine sulphate, DL-propranolol HCl and EGTA (ethyleneglycol-bis (beta-aminoethylether)-N, N'-tetraacetic acid). Polaroid photo-micrographs were taken at varying intervals before and after the addition of drugs to the perfusate. The magnitude of contraction was calculated

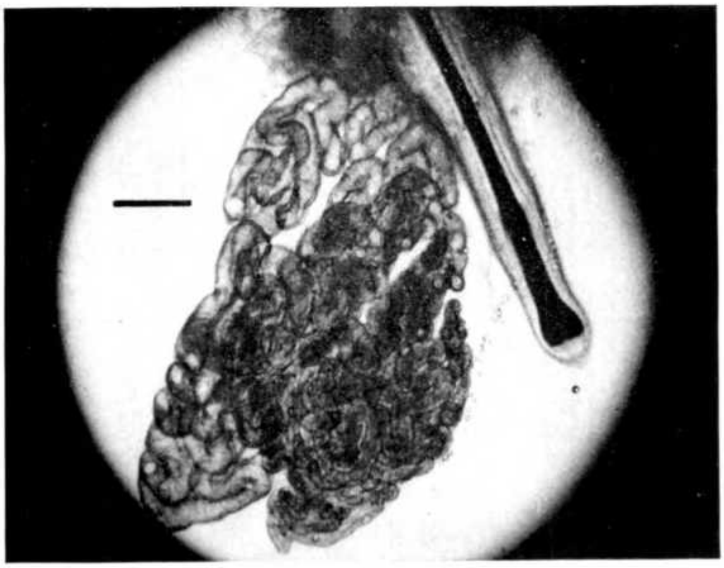

FIGURE 1. Isolated human axillary apocrine glands. Two apocrine glands are attached to this hair follicle. A few more glands attached to the same hair follicle had been already removed during dissection. The short segments of the duct are embedded in the collagen fibres and cannot be seen. The bar indicates 500 μm.

by the formula: 100 $(1\text{-}L_2/L_1)$=percentage contraction. Thus the changes were expressed as a change in the original length of the tubule in the axial direction, where L_1 and L_2 represent the tubular lengths before and after application of drugs.

RESULTS

Figure 2 is an illustrative example of tubular contraction after application of 5×10^{-6}M phenylephrine, an alpha-adrenergic agonist. A persistent contraction without pulsatile or peristaltic movement was observed as long as phenylephrine was perfused. Under microscopic observation, the maximal contraction was reached within 10 s of the drug application. Cessation of drug perfusion and the subsequent washing with KRB resulted in a slow but complete relaxation of the tubule. Adrenaline behaved like phenylephrine in inducing tubular contraction (Fig. 3). However, isoproterenol, a beta-adrenergic agonist, failed to produce tubular contraction, but the subsequent addition of phenylephrine (5×10^{-6}M) to the isoproterenol-containing perfusate did induce the tubular contraction although to a slightly lesser extent than when phenylephrine was perfused alone (Fig. 4). The effect of phenylephrine was completely blocked by the addition of phentolamine (10^{-5}M), an alpha-adrenergic antagonist; however, this was not the case when propranolol (10^{-5}M), a beta-adrenergic antagonist, was added to the phentolamine-containing perfusate. Phenylephrine did not induce tubular contraction when Ca^{++} in the perfusate was sequestered by the addition of 5mM EGTA. Acetylcholine (5×10^{-6}M) was without effect on tubular contractions but a higher concentration of acetylcholine (1×10^{-4}M), which can never be achieved physiologically, did show a minor contractile effect. Table 1 summarizes the results of all the observations. The contraction was

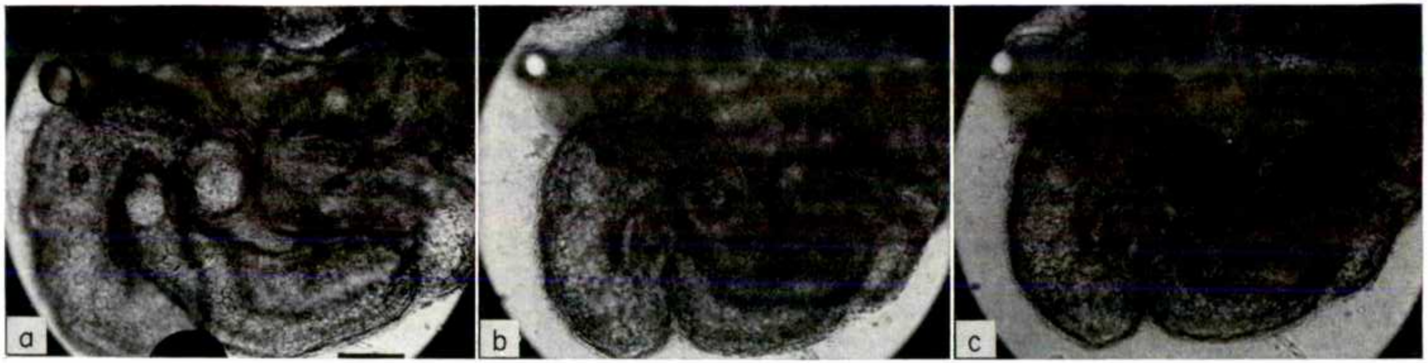

FIGURE 2. A portion of an isolated human apocrine gland before stimulation (a), 10 s (b) and 5 min (c) after start of perfusion with 5×10^{-6} M phenylephrine. Note the persistent contraction of the tubule in (b) and (c) as shown up by the increased area of the white background. No pulsatile or peristaltic contraction was observed under the microscopic observations. The bar in (a) represents 100 μm.

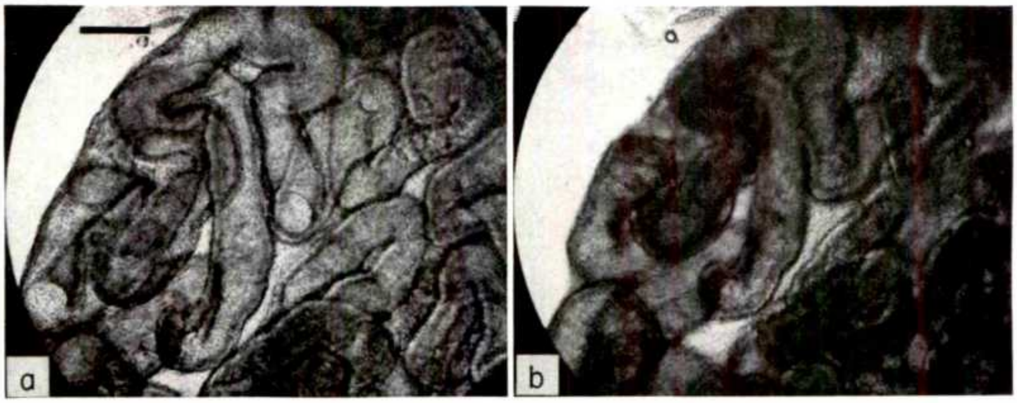

FIGURE 3. Effect of adrenaline on myoepithelial contraction, (a) before, and (b) after perfusion with 5×10^{-6}M adrenaline. This is the same gland as shown in Fig. 1. The bar in (a) represents 200 μm.

TABLE 1. Estimate of percentile contraction of human apocrine myoepithelium to various agents

Drugs (M)	Number of Observations	Percentile contraction* (% ± s.e. mean)
Phenylephrine (5×10^{-6})	7	$23 \pm 2{\cdot}6$
Phenylephrine (5×10^{-6}) + Phentolamine (10^{-5})	4	0 ± 0
Phenylephrine (5×10^{-6}) + Propranolol (10^{-5})	5	$22 \pm 3{\cdot}0$
Phenylephrine (5×10^{-6}) + Atropine (10^{-5})	4	$20 \pm 3{\cdot}4$
Phenylephrine (5×10^{-6}) + Isoproterenol (5×10^{-6})	4	$15 \pm 2{\cdot}8$
Phenylephrine (5×10^{-6}) + EGTA (5×10^{-3})	4	0 ± 0
Phenylephrine (5×10^{-6}) + Acetylcholine (5×10^{-6})	4	$14 \pm 2{\cdot}7$
Adrenaline (5×10^{-6})	4	$20 \pm 2{\cdot}3$
Isoproterenol (5×10^{-6})	2	0
Isoproterenol (10^{-6})	4	0 ± 0
Acetylcholine (5×10^{-6})	6	0 ± 0
Acetylcholine (10^{-4})	4	$8 \pm 1{\cdot}2$

* Percentile Contraction = $100(1 - L_2/L_1)$%, where L_1 and L_2 are respectively the length of tubular segments before and after addition of drugs.

quantified by measuring the tubular length between two arbitrarily chosen landmarks on the tubules. It is clear that alpha-adrenergic stimulation consistently induced tubular contraction, i.e. both phenylephrine and epinephrine caused contraction but acetylcholine and isoproterenol did not. Furthermore, the effect of phenylephrine was blocked by phentolamine but not by propranolol.

DISCUSSION

The present photomicrographic study has shown that isolated segments of the human axillary apocrine sweat glands contract by as much as 23% of the resting length (i.e. the tubule shortens to

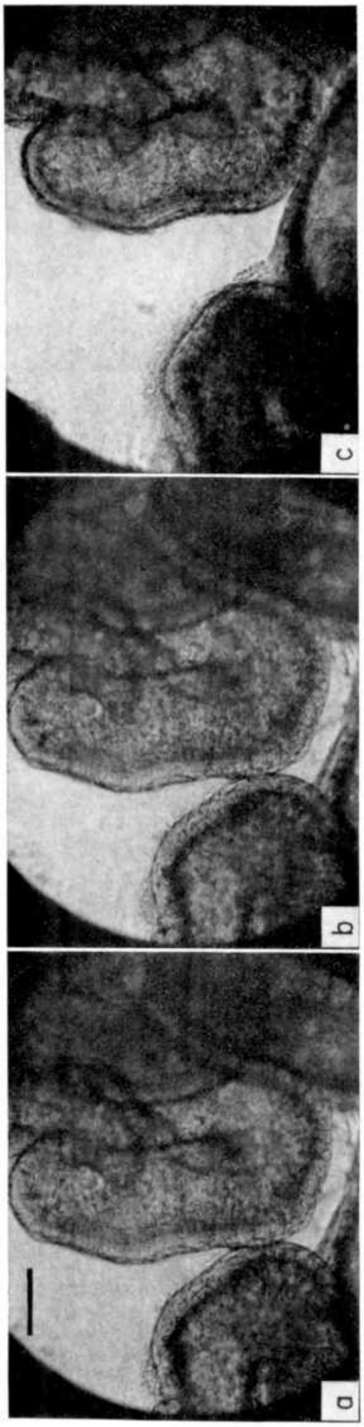

FIGURE 4. A portion of an isolated gland before stimulation (a), after perfusion with 5×10^{-6}M isoproterenol (b) and after perfusion with isoproterenol with phenylephrine (5×10^{-6}M) (c). Contraction is seen only in (c). The bar in (a) represents 100 μm.

77% of the resting length due to contraction) in response to adrenergic drugs. The photomicrographic analysis of tubular contraction corresponds to the measurement of the isotonic contraction under no imposed resting tension. In previous studies from the author's laboratories (Sato, 1977; Sato *et al.*, 1979), an identical photomicrographic method as well as the actual measurement of contractile force by means of a strain gauge transducer were shown to give results that were qualitatively comparable to each other regarding the pharmacological responsiveness of the myoepithelial contraction of the isolated eccrine sweat glands. Therefore, we assume that the present photographic analysis also provides at least a qualitative measurement of the pharmacological responsiveness of the apocrine myoepithelium. The apocrine secretory tubule consists of a single layer of secretory cells and an incomplete layer of myoepithelial cells which are interposed between the secretory cells and the basal lamina (Ellis, 1967). The myoepithelial cells of the apocrine gland are very similar to those of the eccrine sweat glands (Ellis, 1967). Since the apocrine secretory cells do not contain a well-developed cytoskeletal system that can totally explain the observed strong tubular contraction and since the myoepithelium is well developed and shows ultrastructural evidence of contraction after stimulation with adrenaline injection *in vivo* (Bell, 1974), it may be safe to conclude that tubular contraction as observed in the present study represents at least in a large part the myoepithelial contraction.

As summarized in Table 1 the apocrine myoepithelium responds to alpha-adrenergic stimulation because the contractile response was induced with phenylephrine, which was blocked by an alpha-adrenergic antagonist, phentolamine, but not by the beta blocker, propranolol or the anticholinergic agent, atropine. Furthermore, isoproterenol, a beta-adrenergic agonist, failed to induce myoepithelial contraction. Of interest is the effect of acetylcholine; a lower concentration, 5×10^{-6}M, which may be within the range of physiologically achievable concentrations, did not produce myoepithelial contraction whereas 10^{-4}M acetylcholine, a concentration which can never be achieved physiologically, showed a minor contraction. Such a concentration-dependent dual action of acetylcholine is well documented in vascular smooth muscle (Vanhoute, 1978) where a lower concentration of acetylcholine relaxes but a higher concentration constricts the isolated vascular smooth muscle preparations. In the presence of acetylcholine (5×10^{-6}M), the stimulatory effect of phenylephrine on the apocrine myoepithelial contraction was slightly depressed, i.e. from 23% to 14% contraction ($P = 0{\cdot}05$).

The requirement for extracellular Ca^{++} was also observed in the apocrine myoepithelium (Table 1), indicating that the stimulus-contraction coupling, as triggered by the alpha-adrenergic stimulation, is involved in this exocrine myoepithelium as in other smooth muscle preparations. We have previously shown (Sato, 1977; Sato *et al.*, 1979) that the eccrine myoepithelium responds exclusively to cholinergic stimulation; neither alpha- nor beta-adrenergic drugs induced eccrine myoepithelial contraction in isolated monkey and human (unpublished) sweat glands. It is interesting to note that although adrenergic drugs are capable of inducing eccrine sweat secretion, the eccrine sweat gland is a predominantly cholinergic secretory apparatus. In contrast, the pharmacological control of human apocrine secretion still remains to be delineated. If myoepithelial contraction is an integral part of the overall secretory processes *in vivo* and the pharmacological responsiveness of the myoepithelium coincides with that of the secretory cells, as in the eccrine sweat gland, then one might be tempted to speculate that apocrine sweat secretion is primarily an adrenergically-controlled process because of the alpha-adrenergic nature of the apocrine myoepithelial contraction. Indeed the classical study by Shelley & Hurley (1953) indicated that the apocrine secretion is induced only by adrenaline but not by pilocarpine. However, Aoki (1962) later challenged this view by demonstrating that the milky apocrine sweat can be induced around the hair follicle following intradermal injection of pilocarpine as well as adrenaline in man. A recent preliminary experiment by the authors (Sato & Sato, 1979) on isolated single human axillary apocrine glands has demonstrated that the apocrine glands indeed respond to both cholinergic and adrenergic agents, the former being a stronger stimulant of secretion than the latter and furthermore beta-adrenergic stimulation is generally more potent than the alpha counterpart. If this preliminary observation is ultimately proven to be the case, we then face the problem of dissociation in pharmacological responsiveness between the secretory cell function and the myoepithelial contraction. In the eccrine sweat gland, we have already proven (Sato *et al.*, 1979) that myoepithelial contraction without accompanying secretory function by the secretory cells, as in the case of K^+-induced myoepithelial contraction, failed to push out any appreciable amount of preformed sweat into the sweat collection pipette in an *in vitro* cannulated sweat gland preparation. This observation is readily understandable because the luminal space in the eccrine sweat gland is extremely narrow before and even during sweat secretion (Sato, 1977) and thus the amount of preformed sweat retained in the luminal space is negligible. We have therefore discarded the classical concept that the myoepithelial contraction contributes to the eccrine secretory function through its pumping action (Sato *et al.*, 1979). As already discussed in the introduction we now maintain that the primary function of the eccrine myoepithelium is to provide structural support for the secretory epithelium which is capable of withstanding the luminal hydrostatic pressure as great as 500 mmHg in the case of acute poral occlusion (Schulz, 1969). In contrast, the lumen of the apocrine secretory coil is widely dilated and thus contains a considerable amount of preformed sweat before stimulation. The size of apocrine gland differs widely according to the individual and even within the same individual. According to our preliminary estimate, the length of the secretory coil ranges from 5 to 25 mm and the diameter ranges from 80 to 120 μm. Usually several apocrine glands open into a single hair follicle in the human axilla. Thus in the lumen of an intermediate-sized apocrine gland (assuming 15 mm in length and 100 μm in luminal diameter) there is approximately 120 nl of preformed apocrine sweat. During maximal stimulation of the apocrine gland with the alpha-adrenergic agonist, phenylephrine, the tubule contracts by approximately 20% in length (see Table 1) but changes in the luminal diameter were not consistently observed. Thus we can easily estimate that 25 or 30 nl of apocrine sweat can be ascribed to myoepithelial contraction alone. In fact, many, if not all, apocrine glands show a much higher secretory rate for a prolonged period of time in response to acetylcholine and isoproterenol *in vitro* (Sato & Sato, 1979) and the fact that phenylephrine is the least effective stimulant of apocrine sweat secretion leads to a conclusion that, even in the apocrine sweat gland, the pumping action of the myoepithelium plays only an auxiliary role in the overall secretory process. In other words, apocrine myoepithelial contraction may contribute to the initial, transient expulsion of axillary sweat occurring in response to emotional stimuli, but as in the eccrine sweat gland, the apocrine sweat gland is reported to be able to withstand a luminal hydrostatic pressure of as much as 225 mmHg (Shelley & Hurley, 1953). If so, the provision of structural support may be the more important role assigned to the apocrine myoepithelium. Furthermore, the selective response of the apocrine myoepithelium to the alpha-adrenergic stimulation combined with the responsiveness of the secretory cells to both cholinergic and adrenergic stimulation suggests that the dual innervation of the apocrine gland by both cholinergic and adrenergic fibres is the physiological control mechanism of apocrine secretion in man. This contention is supported, if not proven, by observations that both catecholamine-containing and cholinesterase-positive nerve fibres are present around the human axillary apocrine glands (Uno, 1977).

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Serum steroid sulphates in ichthyosis

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SUMMARY

Serum concentrations of pregnenolone sulphate, dehydroepiandrosterone sulphate and 5-androstene-3β,17β-diol sulphate were measured by radioimmunoassays in twelve patients with various types of ichthyosis. In X-linked ichthyosis ($n = 5$), ichthyosis vulgaris ($n = 5$) and lamellar ichthyosis ($n = 1$), steroid sulphates were not significantly higher than in the control subjects. In one baby with ichthyosiform erythroderma and associated deafness serum 5 -androstene-3β,17β-diol sulphate concentration was about 60 fold higher (31 μg/ml) than the mean of the control children. The other steroid sulphate levels were normal in this baby.

These results indicate that it is not possible to demonstrate the steroid sulphatase deficiency in X-linked ichthyosis by determining blood steroid sulphates. The cause of the high 5-androstene-3β,17β-diol sulphate concentration in the baby with ichthyosiform erythroderma needs further evaluation.

Recently Koppe *et al.* (1978) demonstrated deficiency of arylsulphatase c in the skin of three infants who later developed ichthyosis of the X-linked type. Adults with X-linked ichthyosis had the same enzymopathy. Shapiro *et al.* (1978a) could not demonstrate steroid sulphatase activity in cultured skin fibroblasts from infants with X-linked ichthyosis. Later, Shapiro *et al.* (1978b) showed that patients with X-linked ichthyosis from four countries had steroid sulphatase deficiency. In ichthyosis vulgaris and lamellar ichthyosis steroid sulphatase activity was normal in cultured fibroblasts. It was evident that in all patients with X-linked ichthyosis skin fibroblasts were unable to hydrolyse steroid sulphates. To see if steroid sulphatase deficiency can be demonstrated by analysing steroid sulphates from blood, we studied serum concentrations of pregnenolone sulphate, dehydroepiandrosterone sulphate and 5-androstene-3β,17β-diol sulphate in four types of ichthyosis.

The following systematic names are given to steroids referred to by trivial names: pregnenolone, 3β-hydroxy-5-pregnen-20-one; dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one.

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MATERIALS AND METHODS

Patients and control subjects

Altogether twelve patients with various types of ichthyosis were studied. Each patient was examined by an experienced dermatologist and their disease was classified on the basis of clinical appearance, histological criteria and family history. Five patients were diagnosed as having X-linked ichthyosis, five as having ichthyosis vulgaris, one as having lamellar ichthyosis and one as having ichthyosiform erythroderma, who was also deaf. Control subjects were normal adults ($n = 8$) and children ($n = 15$).

Samples

Blood samples were taken from the antecubital vein into glass tubes. Serum was separated by centrifugation and stored at −20°C until analysed.

Steroids and chemicals

Non-radioactive reference steroids were purchased from Ikapharm, Ramat-Gan, Israel or Steraloids, Inc., Pawling, N.Y., U.S.A. Radioactive steroids were obtained from the Radiochemical Centre, Amersham, U.K. Lipidex-5000™ (hydroxyalkoxypropyl Sephadex) was purchased from Packard-Becker, B.V., Chemical Operations, Groningen, The Netherlands.

Analytical procedure

Serum, 100 μl, was diluted with 0·9 ml of water and extracted twice with 1 ml of ether–ethyl acetate 90:10 (v/v). The aqueous layer containing steroid sulphates was separated and dried under a nitrogen flow. Proteins were denaturated by dissolving the residue in 5 ml of ethanol. Precipitated proteins were centrifuged and the supernatant was removed, dried and solvolysed in 10 ml of ethyl acetate previously equilibrated with 100 μl of 2 M sulphuric acid (Vihko, 1966). This procedure liberated the steroids from their sulphate groups. The steroids were further purified and fractionated on Lipidex-5000™ microcolumns using the solvent system petroleum ether–chloroform 96:4 (v/v). To assess chromatographic separation and recovery, tritiated tracers of the steroids were used in each chromatographic run. Steroid radioimmunoassays were carried out essentially as published earlier (Apter *et al.*, 1976).

RESULTS

In Table 1 serum pregnenolone sulphate, dehydroepiandrosterone sulphate and 5-androstene-3β,17β-diol sulphate concentrations are given in control subjects (adults, $n = 8$; children, $n = 15$) and in patients with X-linked ichthyosis ($n = 5$), ichthyosis vulgaris ($n = 5$), lamellar ichthyosis ($n = 1$) and ichthyosiform erythroderma ($n = 1$). In the groups studied, dehydroepiandrosterone sulphate concentration was usually the highest, followed by 5-androstene-3β,17β-diol sulphate and pregnenolone sulphate. In normal children and the baby with ichthyosiform erythroderma, 5-androstene-3β,17β-diol sulphate was quantitatively the main steroid analysed. There were no statistically significant differences in serum steroid sulphate concentrations between the controls and the different types of ichthyosis including the X-linked form. A very high 5-androstene-3β,17β-diol sulphate concentration (31 μg/ml) was observed in the newborn baby with ichthyosiform erythroderma. In this patient the 5-androstene-3β,17β-diol sulphate concentration, in repeated determinations, was about 60 fold higher than the mean of the normal children. The other steroid sulphate concentrations were within normal limits.

TABLE 1. Serum concentrations of steroid sulphates in normal subjects and in patients with X-linked ichthyosis, ichthyosis vulgaris, lamellar ichthyosis and ichthyosiform erythroderma. Values are given as ng of the free steroid in 1 ml serum

Steroid			Pregnenolone sulphate	Dehydroepi-androsterone sulphate	5-Androstene-3β, 17β-diol sulphate
Control adults (n = 8)					
Mean			66	851	324
S.d.			36	540	126
Control children (n = 15)					
Mean			Not analysed	118	488
S.d.				126	581
Patients	Sex	Age (years)			
X1	M	19	95	2150	298
X2	M	9	31	378	69
X3	M	15	44	897	197
X4	M	22	131	2232	596
X5	M	22	136	2332	173
Mean			87	1598	267
S.d.			48	898	201
V1	M	47	36	281	82
V2	F	15	125	1314	156
V3	M	18	57	1131	337
V4	F	19	91	2422	579
V5	F	26	62	1494	253
Mean			81	1328	281
S.d.			40	768	192
L1	F	5	13	34	11
E1	M	Newborn	149	41	31430

X = X-linked ichthyosis; V = ichthyosis vulgaris; L = lamellar ichthyosis; E = ichthyosiform erythroderma.

DISCUSSION

Shapiro *et al.* (1978a, b) have demonstrated a consistent steroid sulphatase defect in cultured fibroblasts from patients with X-linked ichthyosis but not in ichthyosis vulgaris or lamellar ichthyosis. We were interested to see whether blood steroid sulphate determinations have diagnostic value in ichthyosis. In X-linked ichthyosis, where steroid sulphates are not hydrolysed by skin fibroblasts, it was expected that steroid sulphates would concentrate in blood. However, in this study, we were unable to show any difference in steroid sulphate concentrations between controls, X-linked ichthyosis, ichthyosis vulgaris or lamellar ichthyosis. These findings may indicate that steroid sulphates are effectively excreted via their normal routes, biliary and urinary tracts, or that they are hydrolysed by cells other than skin fibroblasts. It is not known if the synthesis of steroid sulphates is reduced in X-linked ichthyosis.

The compounds analysed, pregnenolone sulphate, dehydroepiandrosterone sulphate and 5-androstene-3β,17β-diol sulphate, are some of the main steroid sulphates in human blood (Vihko, 1966; Jänne *et al.*, 1969). In this study the steroids were determined by radioimmunoassay and the concentrations were very similar to earlier results from normal human plasma when the assays were carried out by gas–liquid chromatography (Vihko, 1966; Jänne *et al.*, 1969). In the newborn with ichthyosiform erythroderma and deafness, the serum 5-androstene-$3\beta,17\beta$-diol sulphate concentration was very high in repeated determinations, but the other steroid sulphate levels were normal. As shown before (Laatikainen, Peltonen & Nylander, 1973), serum 5-androstene-$3\beta,17\beta$-diol sulphate concentration is normally at a considerably lower level after birth than in this patient. In liver diseases plasma concentrations of reduced sulphated steroids like 5-androstene-$3\beta,17\beta$-diol sulphate are higher than the concentrations of the corresponding keto compounds like dehydroepiandrosterone sulphate (Sjövall & Vihko, 1966). It remains to be elucidated whether the high 5-androstene-$3\beta,17\beta$-diol sulphate blood concentration is due to steroid sulphatase deficiency or some other cause, e.g. hepatopathy, reported previously in this disease (Desmons, Bar & Chevillard, 1971).

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The 'dry' non-eczematous skin associated with atopic eczema*

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SUMMARY

The non-eczematous but 'dry' skin of twelve patients with eczema has been studied and contrasted with skin from similar sites in control subjects of similar age and sex and with patients with autosomal dominant ichthyosis. The areas investigated were examined histologically and by techniques for assessment of the stratum corneum structure and function. The 'dry' skin sites demonstrated increased intracorneal cohesion, increased total epidermal thickness, patchy parakeratosis and in places slight hypergranulosis. It is concluded that the dry skin condition of patients with atopic eczema is not autosomal dominant ichthyosis and is probably eczematous in nature.

The pathogenesis of atopic eczema is as much a mystery today as it was a decade ago. It is clear that a substantial proportion of the epidermal changes responsible for the clinical signs of the disease are a result of the injuries sustained by persistent and vigorous scratching. It also seems likely that at least a proportion of the sensation of itching and resultant scratching is associated with the 'dry' skin that occurs in the apparently non-eczematous areas of many patients with atopic eczema (DSAE). It should be noted that in this context the term 'dry' is used because of the observed scaling and the apparent decrease of the scaly appearance after the application of water. We have not been able to trace any evidence indicating that the water content of 'dry' skin is reduced. DSAE is more pronounced in winter (Rajka, 1975); it leads to decreased alkali resistance (Burckhardt & Dorta, 1957) and can itself predispose to eczematization. Clinical practice suggests that the 'dryness' of the skin is an important component of the atopic diathesis, and indeed appears to dominate the clinical picture in some patients. In one series there was a 46% incidence of personal history of atopy among 169 patients with ichthyosis vulgaris (Wells & Kerr, 1966). DSAE is frequently referred to as 'ichthyosis vulgaris', implying that the abnormality is the same as, or similar to, autosomal dominant ichthyosis (ADI). The reasons for

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our believing that this may not be the case include differences in body distribution, differences in clinical appearance, differences in inheritance and differences in histology. ADI is more prominent over the extensor aspects, and over the legs the scales tend to be larger and more adherent.

Flexural areas usually remain free of scales. In contrast DSAE affects most of the body indiscriminately and the flexures if not actively eczematous are often markedly 'dry'. Follicular hyperkeratosis over the lateral aspect of the trunk is a common early finding in atopic dermatitis (Ofuji & Uehara, 1973); in ADI the follicular hyperkeratosis seen may be over the extensor surfaces of the extremities. Accentuation of palmar and plantar markings is seen both in ADI and in DSAE.

ADI is characterized by large shield-like lamellae or opaque bran-like scales which may have a 'pasted on' appearance, while DSAE is dominated by fine scaling and a resultant 'roughness'.

ADI is inherited as an autosomal dominant trait so that it is common for one of the parents and indeed several family members to be affected. The inheritance of atopic dermatitis, however, is not unequivocally dominant and the inheritance of DSAE is even more uncertain.

In the follicular, clinically 'ichthyotic' papules associated with atopic dermatitis there is spongiosis and vesicle formation at the upper portion of the follicles with a perifollicular inflammatory infiltrate (Ofuji & Uehara, 1973). In ADI, however, there is hyperkeratosis with a thin or absent granular layer, and the dermis is normal.

Our aim in this study has been to characterize the features of DSAE and contrast this condition with ADI, using histological methods and newly developed techniques for the assessment of the stratum corneum (SC).

MATERIALS AND METHODS

Twelve patients with DSAE, who have given their informed consent, were included. They all satisfied the following criteria: (1) aged 15–24 years; (2) typical clinical picture of atopic eczema with predominantly flexural involvement; (3) history of persistent 'dry' skin; (4) elevated serum IgE level; (5) positive family history of atopy; (6) presence of white dermographism; (7) absence of eczema over the outer aspect of the upper arm.

Patients were instructed not to apply any preparation to the site to be studied in the 2 weeks before investigation. In all cases the area of skin studied was the outer aspect of the upper arm, which in the patients was considered by both patient and examiner to be 'dry'. The degree of 'dryness' at the site studied was recorded on a subjective clinical scale (Table 1) by the same examiner. Eleven individuals with localized and minor skin disorders (such as naevi), who were matched for age and sex, were also studied as controls.

In each subject a 4 mm punch biopsy was taken from the outer aspect of the upper arm; one half of the biopsy specimen was fixed in 10% formalin for routine histological preparation, while the other

TABLE 1. Dry skin subjective score

	Score
Normal skin	1
Examination normal but symptom of dryness	2
Skin looks normal but feels rough	3
Skin looks and feels rough	4
Very rough skin in almost all areas	5

half was frozen prior to cryostat sectioning. Using an eyepiece graticule, mean epidermal thickness was directly measured (μm) as well as determined in terms of cell numbers at twenty random sites allowing estimation of mean keratinocyte height (MKH). The ratio of the length of the basal cell layer to granular cell layer (B/G ratio) was measured using a projection technique. The thickness of the stratum corneum in numbers of horn cells was determined at twenty random sites from cryostat sections after staining using the method of McKenzie & Linder (1973).

The properties of the stratum corneum were assessed using the following techniques:

(1) Forced desquamation ('scrub technique'—Nicholls & Marks, 1977), giving a direct measurement of desquamation after a controlled rotational stress to the skin surface with a blunt perspex 'blade'. The maximum diameter (μm) of the desquamated cells was also assessed by means of a microscope graticule.

(2) 'Cohesography', giving a direct assessment of intracorneal cohesion (in g) in the vertical dimension (Nicholls & Marks, 1977).

(3) Surface contour analysis (employing a surfometer) of skin surface biopsies. The area is measured under a 10 cm length of curve (cm^2), giving an indirect assessment of intracorneal cohesion (Marks & Pearse, 1975).

(4) Transepidermal water loss was assessed using an evaporimeter similar to that described by Nilsson (1977) in g/m^2/h.

(5) Stratum corneum impedance was measured using a modified Wheatstone bridge technique similar to that employed by Lawler, Davis & Griffith (1960).

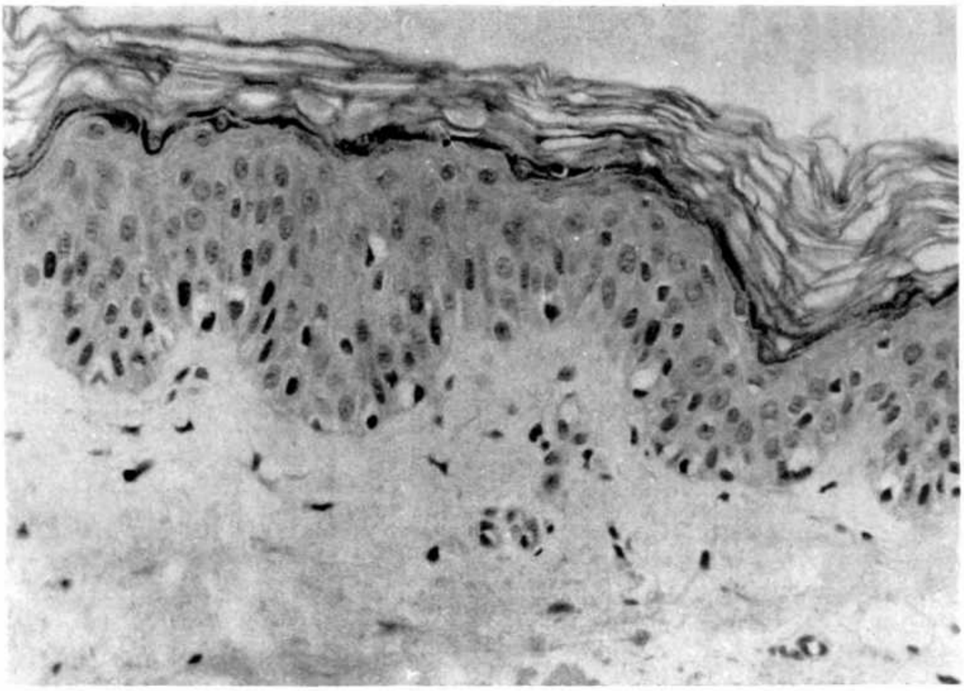

FIGURE 1. Photomicrograph of biopsy from area of dry, apparently non-eczematous skin showing slight epidermal hyperplasia and hypergranulosis.

RESULTS

Degree of clinical 'dryness'

On the subjective clinical scale adopted (Table 1), the degree of dryness of the controls was in all cases assessed as 1. The subjects' scores ranged from 2 to 5 (mean 3·8).

Histology

Morphology. There was slight but definite acanthosis with patchy parakeratosis and in places slight hypergranulosis in the biopsies from dry skinned patients; this feature was not seen in the controls. Figures 1 and 2 are typical examples of skin from DSAE patients showing slight hyperplasia, patchy parakeratosis and slight hypergranulosis. In addition, there appeared to be slight patchy vacuolation of the granular layer in some specimens.

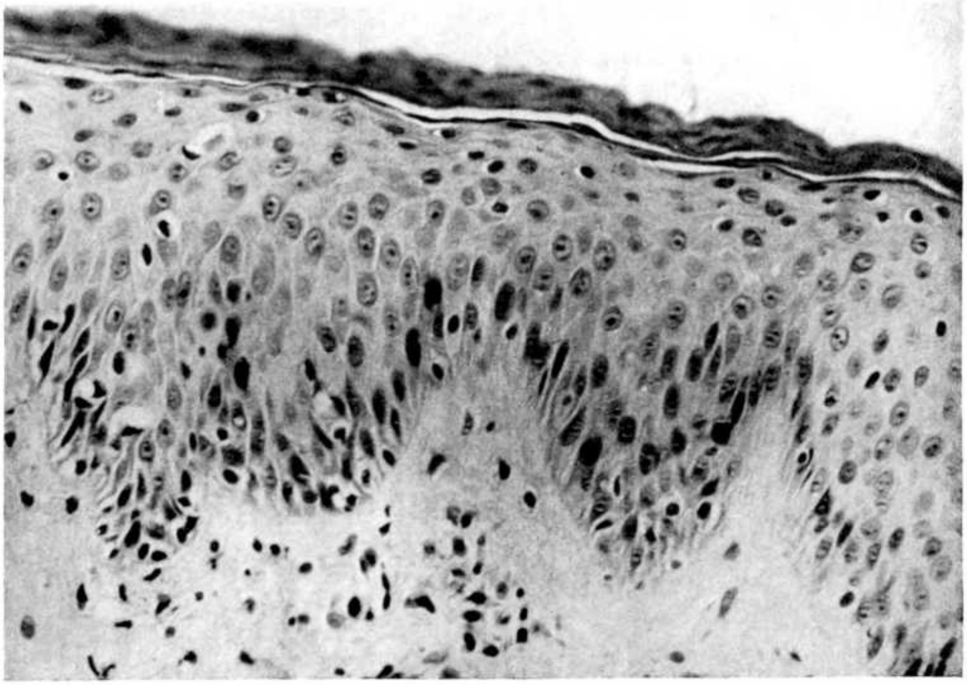

FIGURE 2. There is epidermal hyperplasia and some parakeratosis in this example of apparently non-eczematous but 'dry' skin.

Epidermal measurements (*Table* 2). The mean epidermal thickness, measured both in absolute terms and in numbers of epidermal cells, showed a significant increase in our twelve atopic patients. Mean keratinocyte height in subjects and controls was equal. There was an increase in the B/G ratio in the subjects. There was no significant difference in the thickness of the stratum corneum in terms of cell numbers between the two groups.

TABLE 2. Epidermal measurements

Investigation	Units	Subjects			Controls			Significance (unpaired t-test)
		Number	Mean	± S.d.	Number	Mean	± S.d.	
Mean epidermal thickness	μm	12	84·96	26·53	10	57·22	8·06	$P<0{\cdot}005$
Mean epidermal thickness	Cell numbers	12	7·14	1·98	10	4·88	0·73	$P<0{\cdot}005$
Mean keratinocyte height	μm	12	11·93	1·74	10	11·81	1·33	Not significant
Basal/granular cell ratio		12	1·44	0·42	9	1·18	0·12	$P<0.05$
Stratum corneum cell numbers	Cell numbers	11	13·86	2·31	3	14·00	1·80	Not significant

TABLE 3. Stratum corneum properties in patients and controls

Investigation	Units	Subjects			Controls			Significance (unpaired t-test)
		Number	Mean	± S.d.	Number	Mean	± S.d.	
Scrub technique	Cells/mm^2/10 s	11	56·68 (clumps not included)	29·06	13	72·15	52·67	Not significant
Cohesography	g force	7	102·61	56·55	3	56·67	27·30	Not significant
Skin surface biopsy surfometry	mm^2	12	690·67	235·60	11	605·18	198·01	Not significant
Transepidermal water loss	g/m^2/h	3	11·63	4·99	11	2·88	1·75	$P<0{\cdot}005$
Stratum corneum impedance	Ω	11	1284	436	9	2190	439	$P<0{\cdot}005$

Properties of the stratum corneum (*Table* 3)

(1) Using the 'scrub technique', no significant difference between subjects and controls was found in numbers of single corneocytes dislodged from the surface. However, in four of the subjects in addition to the single cells counted there were large 'clumps' of cells dislodged by the rotating blade. There was no significant difference between the mean sizes of the corneocytes released by the 'scrub technique' from the two groups examined.

(2) Using the cohesograph to measure directly stratum corneum cohesion, we found an increase in the DSAE subjects which, because of the small number of controls who were tested in this study, did not reach statistical significance.

(3) Intracorneal cohesion was also assessed by surfometry of the ruptured internal surface of skin surface biopsies. There were higher readings in the subjects but, once again, these did not reach statistical significance.

(4) There was a large increase in the rate of water loss from the dry skin of three of our patients as measured by the evaporimeter used.

(5) The electrical resistance of the stratum corneum of dry skin was reduced.

DISCUSSION

In our dry skinned atopic patients there was an increase in the thickness of the epidermis, caused by an increase in the numbers of epidermal cells, which themselves were of normal thickness. This finding could be explained by a relative slowing of keratinocyte transit time, or by an increase in the rate of basal cell production. In ADI the mean epidermal thickness is unaltered (Table 4). The increase in the B/G ratio in the dry skin patients parallels the increase in epidermal thickness, and is another reflection of the increase in the population size of the epidermis. The B/G ratio in ADI is unaltered (Table 4). The hypergranulosis seen in DSAE may be part of the overall hyperplasia or may indicate a disturbance in keratinization. Erickson & Kahn (1970) found no difference in thickness of the granular cell layers between those atopic patients with 'dry' skin and those with normal skin. In contrast to our observation of increased thickness of the granular cell layer in dry skin patients they found that normal skin had more layers of granular cells than the skin of atopic patients, whether or not they had dry skin. It is interesting to note that the granular cell layer in patients with ichthyosis vulgaris is virtually absent (Frost & van Scott, 1966).

TABLE 4. Autosomal dominant ichthyosis epidermal measurements and stratum corneum properties

Investigation	Units	Subjects Number	Subjects Mean ± s.d.	Reference
Basal/granular cell ratio	—	6	1·2 ± 0·2	Marks & Dykes (1978)
Mean epidermal thickness	Cell numbers	6	5·2 ± 1·0	Marks & Dykes (1978)
Cohesography	g force	5	153·1 ± 43·8	Nicholls, King & Marks (1978)
Automated cohesography	g force	4	169·5 ± 64·4	Nicholls, King & Marks (1979)

The clinical features of 'dry' skin are related to the physical properties of stratum corneum. The phenomenon of 'clumping' seen in the course of the 'scrub technique' in the DSAE group is also seen in some psoriatic and ichthyotic patients (Marks *et al.*, 1979; Nicholls, King & Marks, 1978) and reflects an increased cohesion between corneocytes, with a resultant tendency of the corneocytes to break away from the surface in large clusters rather than singly. The surfometric findings in skin surface biopsies and the cohesography results support the contention of increased intracorneal cohesion in the stratum corneum of patients with DSAE. Increased cohesion between corneocytes is a feature of diverse patho-physiological processes, including keratinocyte immaturity (as in psoriasis) and in retention hyperkeratosis (as in ichthyosis) in which there is failure of normal desquamation.

Intracorneal cohesion (ICC) in patients with severe ADI has been measured with the manual cohesograph and the results indicated an increase in ICC compared to a normal group (Table 4) (Nicholls *et al.*, 1978). Results obtained subsequently with an automatic cohesograph support the initial findings (Table 4) (Nicholls, King & Marks, 1979). The increased ICC in both ADI and DSAE does not indicate that these disorders have any similarity other than sharing a pathophysiological process which results in scaliness. Hölzle & Plewig (1977) have reported that abnormally small corneocytes are present in dermatitis, and it is interesting to note that we have reported that small corneocytes are also found in many ichthyotic disorders (Marks, 1979). Recent work on corneocyte cell size and stratum corneum function suggests that abnormally small corneocytes may be related to defective barrier function and abnormal cohesion (Nicholls *et al.*, 1979).

The main site of resistance of skin to the conduction of an electric current is the stratum corneum and Campbell *et al.* (1977) have demonstrated an inverse relationship between stratum corneum impedance and the degree of stratum corneum hydration. Lawler *et al.* (1960) reported that patients with atopic dermatitis have a characteristically low impedance, both at involved and uninvolved sites. Our results confirm this and may paradoxically imply that the water content of 'dry' skin is, in fact, increased, as one would expect to occur in areas of eczema.

In active atopic eczema there is a close correlation between transepidermal water loss and the clinical state of activity of the dermatitis (Shahidullah *et al.*, 1969). Our results suggest that the dry skin patients also show an increase in transepidermal water loss at areas of non-eczematized dry skin, and these results parallel the findings of Frost & van Scott (1966), who demonstrated that transepidermal water loss was raised in patients with various ichthyosiform dermatoses. This suggests that although an increase in water content of stratum corneum may be an important factor maintaining skin softness and flexibility (Blank, 1952), a high rate of water flux may be associated with 'dry' skin.

We conclude that the dry skin component of atopic dermatitis is not ADI, and that in the dry skin there are features of disturbed keratinization and epidermal hyperplasia. We have observed increased cohesion between corneocytes, as demonstrated by cohesography and by the finding of 'clumping' in the scrub technique. In addition, we have demonstrated increased water loss from dry skin and decreased electrical impedance. Although the dry areas studied showed no clinical evidence of eczema the clinical and histological features and some of the physical properties found suggest that mild eczematous change may be the root cause of the 'dry' skin in atopic dermatitis.

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Petrolatum: interference with the oxidation of arachidonic acid

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SUMMARY

Microsomal preparations from petrolatum-treated wound skin contained significantly less arachidonic acid-dependent oxidative capacity when compared to activities in untreated wounded skin ($t = 6{\cdot}06$, $P<0{\cdot}001$). Microsomes from both untreated and petrolatum-treated wounded skin produced similar quantities of PGE_2 and $PGF_{2\alpha}$; however, microsomal lipoxygenase activity in petrolatum-treated wounded skin was depressed when compared to that in untreated wounded skin. Microsomal preparations from normal pig skin treated with petrolatum oxidized arachidonic acid at a similar rate to those from untreated control skin.

Increasing quantities of petrolatum progressively inhibited the synthesis of PGE_2 and $PGF_{2\alpha}$ by fetal calf skin microsomes *in vitro* as determined by both the oxidation of arachidonic acid and the quantification of radioactive product.

Topical application of banal compounds that contain lipid substances may therefore alter the cutaneous inflammatory response by local suppression of pathways that generate hydroxy-fatty acids, substances that are known to be chemotactic and pro-inflammatory.

Petrolatum (soft paraffin), a mixture of semisolid hydrocarbons chiefly of the methane series with the general formula, C_nH_{2n+2}, is a colloidal system of non-straight chain solid hydrocarbons and high boiling liquid hydrocarbons, in which most of the liquid hydrocarbons are held inside the micelles. Petrolatum is manufactured by distillation of asphalt-free petroleum followed by decolourization of the product (Schindler, 1961). The clinical benefits of petrolatum were recognized quickly by physicians. Early accolades referred to the treatment of burns, the skin lesions of which are now known to

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be associated with increased release of prostaglandin (PG) (Angaard & Jonsson, 1971). Contemporary clinicians have noted that petrolatum preparations which contain no known pharmacologically active ingredients frequently produce partial or complete resolution of skin inflammation.

Little is known about the pharmacological potentials of topical vehicles. Skin penetration studies are not readily available for most of these substances, such as petrolatum, even though they have been used clinically for generations. If pharmacological effects could be demonstrated, isolation of the active fraction(s) from complex organic mixtures such as petrolatum might not be possible for specific studies.

We hypothesized that decreased skin redness which frequently follows the application of petrolatum is related to suppression of the synthesis or release of pro-inflammatory mediators in skin such as PGE_2 and hydroxy-fatty acids. If this is so, then a factor(s) in petrolatum must penetrate into skin where it inhibits the synthesis of mediator. In this report, we have examined the ability of microsomal fractions prepared from petrolatum-treated normal and wounded pig skin to oxidize arachidonic acid. Our results support the concept that petrolatum or a component of petrolatum can penetrate into damaged skin where it may act as a pharmacologically active material, perhaps by influencing the generation of substances that are important in the inflammatory and regenerative processes in skin.

MATERIALS AND METHODS

Wounding method

White domestic pigs (12–18 lb) were shaved with standard animal clippers and the remaining hairs were removed with a barber's clippers. The skin on both sides of the animal was prepared for wounding by washing with sterile saline. Antiseptics were not used because of their potential effect on the healing process.

Each pig was anaesthetized with sodium pentobarbital (15 mg/kg) i.p. and two parallel wounds, 0·3 mm deep, were made in the paravertebral and thoracic areas with a Castro-Viejo dermatome. Roughly 4 cm separated the wounds. The pigs were housed separately after wounding.

A measured aliquot of petrolatum (USP) was applied to one of the wounded sites and to a selected site of untreated skin daily for 7 days. Earlier studies had shown that both treated and untreated wounds are re-epithelialized within 7 days. The pig was anaesthetized as described above and the areas scrubbed with soap and water to remove adherent petrolatum. Strips of skin were then removed with a Castro-Viejo dermatome from all sites and frozen at −70°C until assayed.

Oxygen monitor studies

The oxidation of arachidonic acid by the various microsomal preparations was determined by measuring oxygen absorption at a constant temperature of $30 \pm 0{\cdot}5$°C. Oxygen concentrations were measured on a Yellow Springs Instrument Company Model 53 oxygen monitor equipped with a Model 5301 bath assembly as reported previously by Smith & Lands (1971, 1972). Immediately before the assay, PG synthetase was prepared by homogenizing aliquots of (1) acetone powder fetal calf skin extract (purified by the procedure of Wallach & Daniels, 1971), or (2) microsomal pellet from hog skin, in 1 ml of 100 mM Tris-HCl buffer, pH 8·5, in a small Dounce ball-type homogenizer. Additions of enzyme preparations alone or with aliquots of a 10% suspension of petrolatum previously sonicated into 100 mM Tris-HCl, pH 8·5, were made through the side of the electrode holder. Immediately thereafter, arachidonic acid (20 μM final concentration) (Sigma, St Louis) was added to the 3·0 ml reaction mixture and the rate of oxygen consumption (μmol O_2 min^{-1}) was determined by continuous measurement of oxygen uptake.

Conversion of ^{14}C-arachidonic acid to radioactive PG

^{14}C-arachidonic acid (specific activity 56·6 mCi/mmol, New England Nuclear, Boston, Mass.) was evaluated for purity in the thin layer system, chloroform:methanol:acetic acid (180:10:10) on silica gel G (type 60) (Merck, Darmstadt, Germany). A homogeneous peak was observed; however, because of low conversion rates to PG, ^{14}C-arachidonic acid was further evaluated for the presence of hydroxy metabolites of arachidonic acid (Ziboh, Vanderhoek & Lands, 1974). An aliquot of ^{14}C-arachidonic acid was treated with diazomethane and the methylated product was chromatographed on silica gel G in the solvent system, chloroform:acetic acid (180:2). Approximately 40% of the commercial product was found to migrate in the area of hydroxy-fatty acid, far removed from the methyl arachidonate region.

^{14}C-arachidonic acid was purified prior to each experiment by silicic acid chromatography. The ^{14}C-arachidonic acid was then stored in a known amount of hexane until use. Immediately after purification, examination of a methylated aliquot by thin layer chromatography revealed less than 1% contaminant.

For determination of the conversion of ^{14}C-arachidonic acid to PGE_2, $PGF_{2\alpha}$ and hydroxy-fatty acid, the standard 2 ml reaction mixture contained: 0·1 μCi ^{14}C-arachidonic acid, 0·55 mM hydroquinone, either 15 mg of acetone powder preparation of fetal calf skin microsomes or an aliquot of microsomal preparation from pig skin, and the presence or absence of 3 mg petrolatum in 100 mM Tris-HCl buffer, pH 8·5. Incubations were carried out for 5 and 10 min at 37°C with shaking and were terminated by the addition of 2·5 vols of chloroform: methanol (2:1). Radioactive PGE_2 and $PGF_{2\alpha}$ were extracted, purified and quantified as previously described (Ziboh, 1973). Hydroxy metabolites of arachidonic acid were separated from unreacted arachidonic acid by methylation and chromatography on silica gel G in the system, chloroform:acetic acid (180:2). Data were analysed as the per cent conversion of radioactive arachidonic acid into the pertinent metabolite and represent the total radioactivity in that fraction divided by the total extractable radioactivity multiplied by 100. Authentic PG, used as standards, was the kind gift of Dr Udo Axen (Upjohn). Protein determinations were by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

RESULTS

The application of petrolatum daily to normal and wounded skin

The once daily application of petrolatum to normal skin produced acanthosis; this observation confirmed earlier work in human skin (Brun, Bujard & Jadassohn, 1956). On the other hand, application of petrolatum to wound inhibited the hyperplastic response normally observed in untreated wound. This inhibition may represent an abrogation of this hyperplastic reaction or a delay in its development since Eaglstein & Mertz (1979) have observed that petrolatum delays wound healing.

Increasing quantities of microsomal preparation purified from normal pig skin consumed increasing quantities of oxygen in the arachidonic acid-dependent oxygen consumption assay, suggesting an enzyme-mediated reaction in the presence of excess substrate. In all experiments that quantified microsomal activity, between 40 and 120 μg of protein were used in the presence of excess substrate.

Arachidonic acid-dependent oxygen consumption by microsomal preparations from petrolatum-treated normal skin was not significantly different from that in microsomal preparations from untreated normal skin (Fig. 1). The application of petrolatum to wound, however, resulted in a microsomal preparation that consumed significantly less oxygen than did that from untreated wound ($t = 6{\cdot}06$, $P < 0{\cdot}001$).

Microsomal preparations from petrolatum-treated wound and untreated wound of three pigs were incubated with radioactive arachidonic acid and the PGE_2 and $PGF_{2\alpha}$ products were isolated to

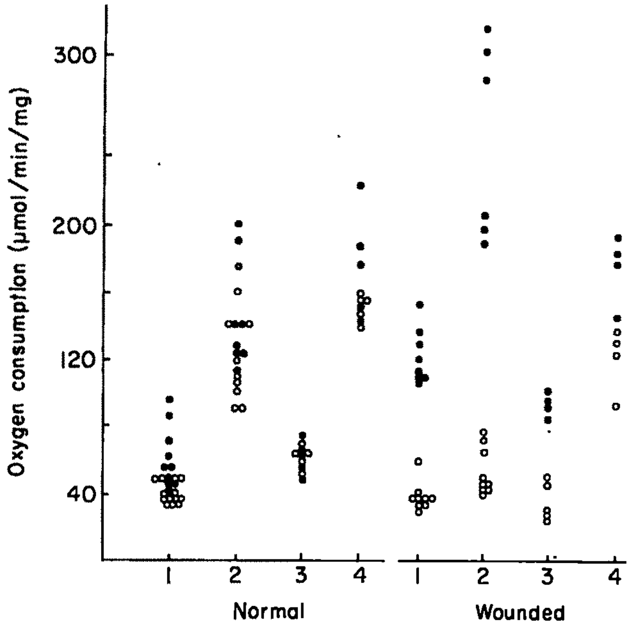

FIGURE 1. The effects of petrolatum on arachidonic acid-dependent oxygen consumption by microsomal preparations from skin of normal and wounded areas. (●) Untreated; (○), petrolatum-treated. The numbers on the abscissa refer to the experiment (pig).

determine if inhibition in the oxygen consumption assay represented true suppression of the formation of PG product and not the inhibition of some other arachidonic acid-consuming activity (such as microsomal lipoxygenase). Microsomal preparations from petrolatum-treated and untreated wound converted similar quantities of arachidonic acid into PG (control wound: 1·0±0·2 and 1·9±0·3; petrolatum-treated wound: 1·1±0·5 and 2·0±0·3, 5 and 10 min incubation periods). Separation of radioactive hydroxy-fatty acid from the same incubation mixtures revealed that preparations from petrolatum-treated wound synthesized less radioactive hydroxy-fatty acid than did those from untreated wound (control wound: 13±2 and 16±2; petrolatum-treated wound: 7·3±2·1 and 9·1±2·0, 5 and 10 min incubation periods). These data suggest that the decreased oxygenation of arachidonic acid by microsomal preparations from petrolatum-treated wound (Fig. 1) most likely reflects decreased activity of a microsomal lipoxygenase.

The effects of petrolatum on fetal calf skin PG synthetase in vitro

The presence of increasing amounts of petrolatum added to the reaction vial inhibited arachidonic acid-dependent oxygen consumption by fetal calf skin microsomes in a linear fashion (Fig. 2). Although oxygen consumption generally reflects PG and hydroxy fatty acid biosynthesis, these data must be interpreted with caution because of the difficult problem of introducing reproducible quantities of petrolatum into the reaction mixture.

Incubation experiments with fetal calf skin microsomes and radioactive arachidonic acid indicated that petrolatum did inhibit the formation of PG by fetal calf skin PG synthetase. The amount of PGE_2 and $PGF_{2\alpha}$ synthesized increased with time, but was diminished by the presence of petrolatum (control: 0·36±0·06 and 0·49±0·08; 1·5 mg/ml petrolatum: 0·17±0·01 and 0·27±0·04, 5 and 10 min incubation periods). Analysis of PGE_2 and $PGF_{2\alpha}$ individually produced similar inhibitory patterns.

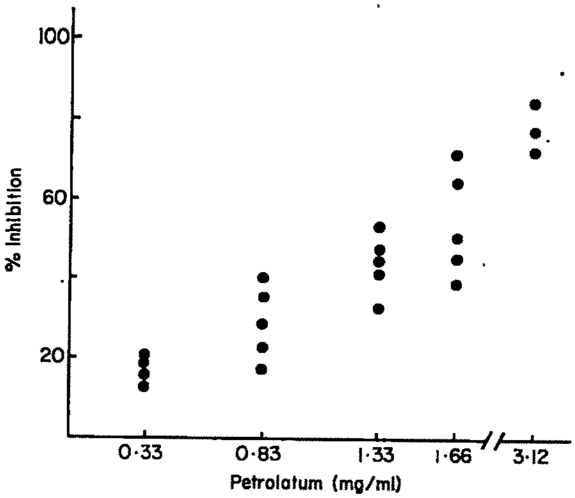

FIGURE 2. Inhibition of arachidonic acid dependent oxygen consumption by petrolatum in microsomal preparations purified from fetal calf skin. Petrolatum was sonicated into the assay buffer. The quantity of petrolatum present in the reaction mixture was calculated by assuming an even dispersion of lipid micelles in the buffer.

The absence of decreased activity in preparations of skin treated with petrolatum *in vivo* suggests that the inhibitory factor in petrolatum detected *in vitro* failed to penetrate wounded skin in sufficient quantities to inhibit PG synthetase.

DISCUSSION

The beneficial effects of a commonly applied 'banal' substance, such as petrolatum, are difficult to explain. Clinical improvement has generally been ascribed to the physical effect of removing scale, crust and debris, a protection from irritants, an allowance of natural recuperative functions to operate, etc. It seems likely, however, that complex biochemical mixtures such as petrolatum, lanolin and mineral oil from organic sources would contain fractions that are active in various biochemical systems.

Scattered reports have indicated that petrolatum exerts definite effects *in vivo*. Application of petrolatum to intact normal skin produces acanthosis (Brun *et al.*, 1956), an observation that was confirmed in our study. Applying petrolatum to a healing wound delayed re-epithelialization (Eaglstein & Mertz, 1979). Application of petrolatum to stripped mouse skin followed by occlusion for 24 h produces marked suppression of the mitotic index when compared to appropriate controls (Tree & Marks, 1975). In this study, at 7 days after wounding, the hyperplastic response seen in control wounds was abrogated in the petrolatum-treated areas. Our data also support the hypothesis that topical petrolatum exerts effects on damaged skin. Numerous lipid materials have been reported to inhibit PG synthetase *in vitro* (Ziboh *et al.*, 1974; Pace-Asciak & Wolfe, 1968), and petrolatum did indeed suppress the activity of an acetone powder extract of fetal calf skin PG synthetase in microsomal preparations prepared from intact skin treated with petrolatum.

We demonstrated decreased oxygenation of arachidonic acid by microsomal preparation from petrolatum-treated wounded pig skin. This decrease probably reflects partial inhibition of a microsomal lipoxygenase. However, the techniques that we have used are limited. The oxidation of arachidonic acid as measured by the oxygen monitor is a non-specific assay which measures the sum of at least two reaction sequences (PG and hydroxy-fatty acid formation). Radionuclide experiments are more precise; however, the products were not confirmed by mass spectrometry.

Recently, Hammerström *et al.* (1979) observed that 12-L-hydroxy-5,8,11,14-eicosatetraenoic acid, a lipoxygenase product, was a major metabolite of arachidonic acid in human and mouse epidermis (little PGD_2, PGI_2 and thromboxane A_2 were synthesized); previously, they had observed that this metabolite was increased in psoriatic plaque when compared to that in control skin (Hammerstrom *et al.*, 1975). Although the function(s) of the lipoxygenase metabolites in skin remain obscure, it is likely that one or more are pro-inflammatory. Topical application of petrolatum suppressed pig skin microsomal lipoxygenase activity *in vivo*, an observation that could explain the often observed suppression of redness in dermatitic skin by petrolatum application. Compounds in vehicles, other than petrolatum, also exert biochemical effects *in vivo* (Komatsu, Asaba & Suzuki, 1979). Further evaluation of these 'banal compounds' in vehicles may reveal other materials capable of penetrating inflamed skin where they may interfere with pro-inflammatory sequences.

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Cutaneous biometrics I. The response of human skin to dimethyl sulphoxide

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SUMMARY

The wealing response of human skin to dimethyl sulphoxide (DMSO) has been quantified. Concentrations of 90%, 95% and 100% were applied for 5 min to circular areas 8 mm in diameter. Wealing was scored on a five-point scale after 10 min.

Marked individual variations were found. Wealing was strongly influenced by body region and was dose-dependent. The intensity of the reaction paralleled that to other irritants and was dependent mainly on thickness and integrity of the horny layer. The DMSO test is a simple, quick way to assess the barrier function of the stratum corneum.

This report is one of a series concerned with the development of simple, non-invasive procedures for assessing the reactivity of human skin quantitatively.

Internists possess a sophisticated technology for evaluating the functional behaviour of organs, such as the heart, liver and kidney. By contrast, externists have very limited means for assessing the physiological capacity of skin in health and disease. Dermatologists lack standardized, clinically feasible methods for determining the normality of the integument. The skin often appears normal when, in fact, it is significantly deranged. We have often been startled by the disparity between the gross and microscopic findings.

The alkali neutralization and resistance tests (Burckhardt, 1947) were designed to detect individuals with an unusual susceptibility to chemical irritants. However, it has proved to be unreliable and has been judged to have little or no clinical value (Vesey & Calnan, 1958; Björnberg, 1974). Transepidermal water loss is a function that provides information on the integrity of the horny layer barrier. Still, many technical problems must be solved before this measurement becomes a routine. A variety of other procedures have similar limitations.

Our goal is to develop simple techniques which evaluate various skin functions *in vivo*. We use the term 'cutaneous biometrics' for tests that yield a quantitative estimate of specific types of physiological reactions.

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MATERIALS AND METHODS

Concentrations

Analytical grade DMSO was prepared in three strengths: 90%, 95%, and 100%, using water as the diluent. Because DMSO is hygroscopic, the solutions must be kept in air-tight bottles. These may be stored for months without loss of potency.

Application

The most convenient way to apply all three strengths simultaneously is to use a plastic block (15 × 3 × 0·3 cm) in which three holes, each 8 mm in diameter, have been drilled (Fig. 1a). Silicone grease (high viscosity grade) is smeared around each well to ensure tight sealing. On the extremities the block may be held firmly in place by flat, rubber tourniquets; elsewhere adhesive tape is suitable. Each well holds

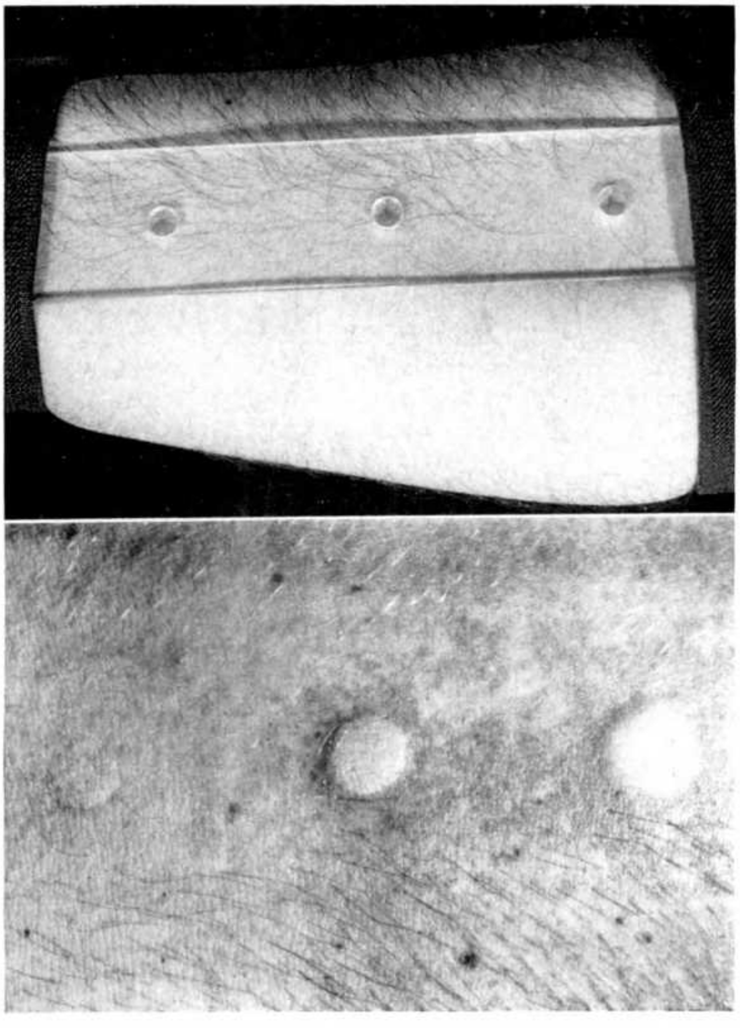

FIGURE 1. (a) Plastic block containing three wells 8 mm in diameter. (b) Weal responses on the forearm of a sensitive subject (from left): 1+ at 90%, 3+ at 95% and 4+ at 100%. Flares are also present.

about 150 μl. A Pasteur pipette is used for filling. Cover slips are placed over the walls to prevent leakage.

For very curved surfaces or where the test area is small (the mastoid, for example), little brass rings, 8 mm in diameter and 3 mm high, can be substituted. These are first dipped into a thin layer of grease and then firmly pressed to the skin. A coverslip is applied after instilling 150 μl of the solution. The skin need *not* be prepared before testing. Defatting with ether or a short wash with soap and water does not affect the result.

Exposure

The exposure time is 5 min. The fluid is then aspirated and the site dried with a cotton-tipped applicator.

Reading

The fully developed weal has a sharp round outline surrounded by an erythematous flare. Follicular weals appear first, later becoming confluent. The weal and flare are graded separately 10 min after aspirating the DMSO as follows: (a) wealing: 1+ discrete follicular weals; 2+ mainly follicular with some merging; 3+ solid weal but only slightly elevated; 4+ solid, tense weal. (b) Flare: the longest and shortest diameters of the flare were averaged.

Subjects

The DMSO test was performed on over 200 male and female college student volunteers, aged 18–30.

RESULTS

Dosage response

The DMSO test was performed on the ventral forearms of thirty-one white subjects. The majority failed to respond to the 90% strength; 1+ and 2+ weals were provoked by the 95% concentration. Neat DMSO regularly produced 3+ to 4+ weals (Fig. 2). Typical weals are illustrated in Fig. 1b.

The diameter of the flare was also dose-dependent. The threshold concentration for erythema was 95%. The 100% concentration always produced significant flares.

Although the magnitude of the weals and flares tended to parallel each, the weal is far less variable and has greater reproducibility. The mean flares for 3+ and 4+ weals were not different statistically ($21 \pm 9{\cdot}0$ versus $24 \pm 9{\cdot}0$). We decided therefore to ignore the flare response in subsequent tests.

Exposure time

Ninety per cent DMSO was applied to the ventral forearms of eight white subjects for 5, 10, 15 and 20 min each. The sites were evaluated at 1, 10, 20 and 30 min after removing the DMSO.

The results are illustrated in Fig. 3. Wealing increases steeply with exposure time, approaching an exponential function with the longer times. The reaction peaks between 20 and 30 min. Because the 10 min reading is only slightly less than the maximum, we adopted the 10 min measurement as the standard.

Regional variations

Figure 4 shows data on fifteen subjects tested on the forehead, upper back, mid-ventral forearm, antecubital fossa, ventral wrist, and lower leg. The forehead was most reactive, the 90% strength giving 4+ weals in almost every case. The ventral aspect of the wrist was least reactive, being generally

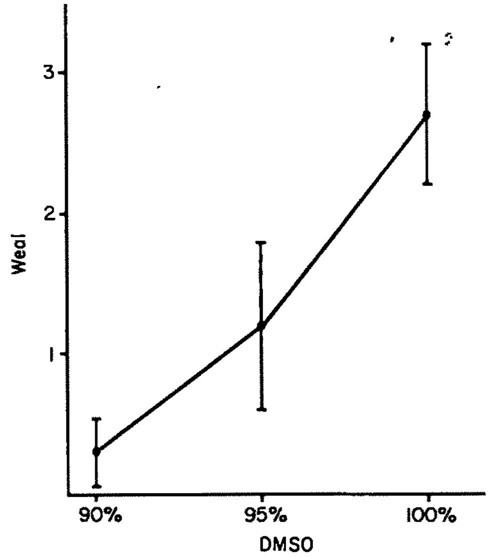

FIGURE 2. Dose-response curve of DMSO weals on the forearm (n = 31).

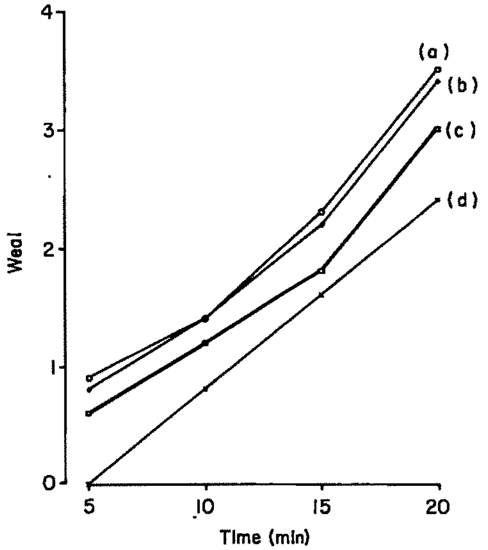

FIGURE 3. Effect of exposure time on wealing to 90% DMSO. Wealing increases with extension of exposure from 5 to 20 min. The reaction peaks at about 20 min. Sites were evaluated at (a) 30, (b) 20, (c) 10 and (d) 1 min after removing the DMSO (n = 8).

unresponsive to 100% DMSO. The rank order of reactivity was: forehead > upper back > antecubital fossa > mid-ventral forearm > lower leg > ventral wrist. When 100% DMSO failed to elicit a response after 5 min, we increased the exposure to 10 and, in some instances, to 20 min. The palm often failed to weal after 30 min. Still, here too, longer exposures were effective.

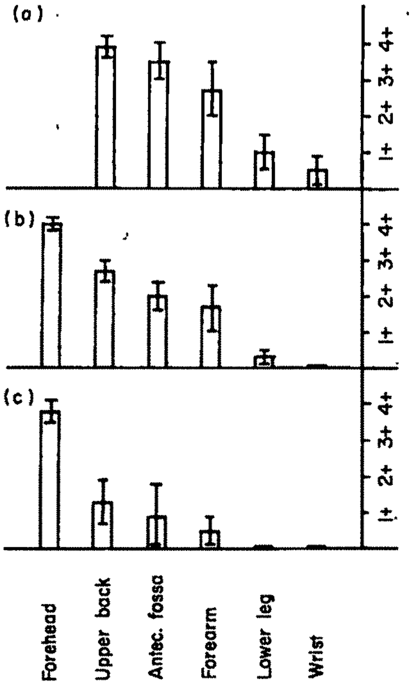

FIGURE 4. Regional variations of the DMSO reaction: (a) 100% DMSO; (b) 95% DMSO; (c) 90% DMSO.

Weal resorption time (clearance)

In six subjects a 4+ weal was induced on the ventral forearm, lower leg, upper back and over the mastoid region. The sites were read hourly for 4 h. The results are shown in Fig. 5.

The mastoid was very sensitive and comparable to the forehead, generally requiring only 5 min of 95% DMSO to produce a full weal. After 1 h the weal had greatly diminished. The skin appeared completely normal in all six subjects after 2 h. In contrast, wealing subsided only gradually in other body regions. The slowest clearance was on the forearm followed by the lower leg and the back. In general, the larger the weal initially, the longer the clearance time.

Individual variations

Figure 6 shows data for the forearm for forty-eight white subjects. Every one reacted to 100% DMSO, almost 45% with a 4+ weal. Six (12%) showed a threshold response of 1+ and another six attained 2+.

With 90% DMSO, thirty-seven of the forty-eight subjects were completely unreactive; eleven barely formed a weal. We wished to know whether a strong (or a weak) response in one body area correlated with a similar response elsewhere. Pilot studies showed this to be the case. For example, five very sensitive subjects with a mean 3·2+ weal to 95% DMSO on the back gave a 2·0+ reaction on the forearm. By contrast, five resistant subjects with a mean weal of 1·2 on the back showed a correspondingly low value on the forearm (0·7). Comparisons between other body regions invariably demonstrated the validity of the principle.

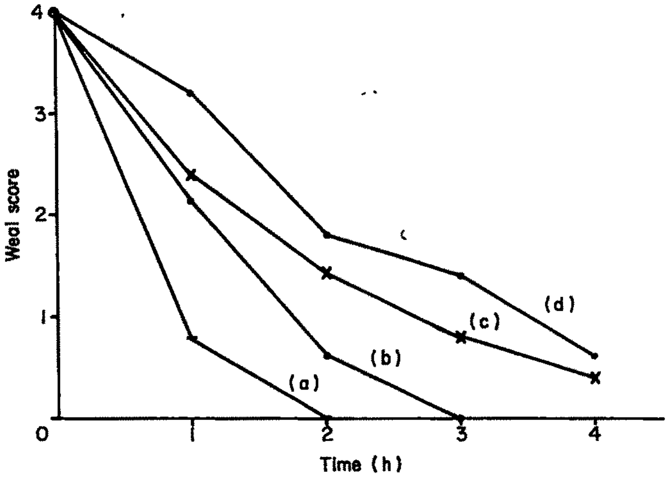

FIGURE 5. Regional variations of weal clearance: (a) mastoid; (b) upper back; (c) lower leg; (d) forearm.

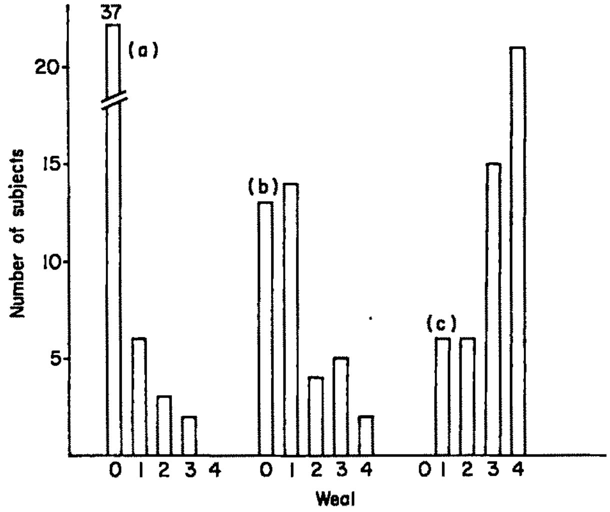

FIGURE 6. Individual variations in DMSO reactivity of the forearm: (a) 90% DMSO; (b) 95% DMSO; (c) 100% DMSO.

Reproducibility

Twelve subjects tested on the ventral forearm were re-exposed 1–20 days later. In no instance was divergence from the original value striking; a one-point difference was observed in a few cases. The mean values were 2·9 for the first test and 3·1 for the second (not significant by Student's *t*-test).

Seasonal variation

The data shown in Fig. 6 were obtained during the cold winter season (November to February). When the same persons were tested again during the hot summer time (June to August), much smaller weals were obtained. During the winter, all subjects reacted to 100% DMSO, 75% of them with a severe 3 or 4+ reaction. In contrast, only 52% reacted with the same intensity during the summer period, and a substantial number, 20%, were completely unresponsive. The difference was statistically significant ($P<0.05$, *t*-test).

Effect of sex

There were no sex differences, either in the summer or in the winter (fifteen females, 3·1±0·9; thirty-three males, 3·0±1·1).

Effect of race

Nineteen white subjects (nine males, ten females) were compared with sixteen black subjects (nine males, seven females) on the ventral forearms. The mean with 100% DMSO was 3·1 (±0·40) for whites and only 1·3 (±0·7) for blacks. This difference was statistically significant ($P<0.05$, *t*-test). Again, there was no sex difference within each race.

Miscellaneous studies

These were carried out on panels of five subjects each with comparable reactivity to DMSO.

Stripping. The skin was stripped 3, 5, or 10 times and to the glistening layer with Scotch tape, followed immediately by exposure to DMSO. Table 1 includes only the wealing scores at the most differentiating DMSO concentration. Even after 3 strips (removing only the desquamating portion) the weals were somewhat larger, definitely so after 5.

Ten strippings converted the weakest reactors to strong ones. After complete removal of the stratum corneum and waiting one hour for the hyperaemia to resolve, 90% DMSO induced a tense weal in every subject.

Scarification. The skin was lightly scarified with a 27 gauge needle in a criss-cross pattern, four

TABLE 1. Reactions to DMSO under various conditions. Means and standard errors at the most differentiating concentrations

Treatment	Normal skin		Treated skin	
Stripping ×3	1·0	(0·5)	1·2	(0·6)
Stripping ×5			1·8	(0·8)
Stripping ×10			3·4	(0·9)
Glistening layer			4·0	(0)
Scarification	0·4	(0·2)	3·4	(0·5)
Hydration (24 h)	0·2	(0·2)	2·0	(0·4)
Hydration (7 days)	0·2	(0·2)	4·0	(0·8)
SLS*	0·8	(0·4)	4·0	(0·7)
Soap wash*	1·0	(0·5)	3·0	(0·7)
Hyperthermia	2·8	(0·3)	1·2	(0·8)
Hypothermia	3·4	(0·6)	2·0	(0·9)
Adrenalin† i.c.	3·8	(0·3)	1·8	(0·4)
Compound 48/80 i.c.	0·3	(0·2)	2·2	(0·4)

*Plain water as control.
†Normal saline as control.

strokes in each direction. Bleeding was avoided. The DMSO reaction was greatly enhanced. Wealing began along the scarifications (Table 1).

Hydration. Squares of wetted non-woven cotton cloth (Webril, Curity) were maintained on the forearm under occlusion with impermeable plastic tape (Blenderm, 3 M Co.) for 1 and 7 days respectively. Exposure to DMSO took place immediately upon removing the dressing and again 24 h later. When the skin was still wet, with both periods of hydration, the DMSO response was the same or slightly reduced compared to normal skin. It should be recalled that DMSO reacts exothermically with water and is strongly hygroscopic. However, allowing an interval of 24 h for the skin to dry out, both hydrated areas showed markedly increased DMSO reactions, the values being greatest after 7 days of hydration (Table 1). Sweating for 15 min in a hot environmental chamber (40°C, 95% relative humidity) had no effect on the DMSO response.

Soap damage. Aqueous sodium lauryl sulphate (SLS) (0·25%) was applied for 24 h on the ventral forearm via aluminium chambers (Duhring chambers), holding 0·1 ml of the solution (Frosch & Kligman, 1979). The DMSO test was performed 3 h after removing the chambers. Although the skin looked normal, the DMSO response was sharply accentuated at the SLS treated sites (Table 1).

The forearms on one side were washed twice daily with Ivory soap (Procter & Gamble Co.) for 3 days, using a soft Webril pad. Each washing was for 30 s followed by a rinse. This caused no visible reaction. The DMSO test was performed on the fourth day. The responses were unequivocally increased on the washed side (Table 1).

Blood flow. Hyperthermia was induced by placing a heating pad (40°C) for 15 min on one forearm with immediate exposure to DMSO. The response on the heated side was slightly less, the mean of scores being 1·2 and 2·8 respectively (Table 1).

Hypothermia was induced by ice cubes applied to the skin for 15 min. The DMSO test performed immediately upon removal showed a slightly decreased reaction (Table 1).

Adrenalin. A 1:1000 solution (0·05 ml) of adrenalin was injected intracutaneously into the forearm. A 2 × 3 cm area of intense vasoconstriction was present 20 min later at which time the DMSO test was performed. The controls were normal skin and skin injected with normal saline. DMSO wealing was greatly reduced by adrenalin (Table 1). Saline did not alter the response.

Compound 48/80. A 0·1% solution of 48/80 in normal saline (0·05 ml) was injected intracutaneously into the forearm of eight subjects. An intense weal with erythematous flare developed within 20 min. The DMSO test was performed 3 h after injection on previously wealed but normal appearing skin. The DMSO reaction was greatly increased by two score points in comparison to a saline injected site (Table 1).

Repeated DMSO exposure. The same skin site was exposed at hourly intervals five times in five subjects. The reaction increased with every exposure. A weak responder turned into a strong one after the third or fourth exposure. The mean for the 100% DMSO reaction increased from 1·5 ± 0·6 from the initial test to 4·0 ± 0 after the fifth test (0 vs 1·3 ± 0·7 for 90% DMSO).

After 24 h marked redness and slight induration were present indicating tissue damage.

Psoriatic skin

Seven patients with plaque type chronic psoriasis were tested using nearby uninvolved skin after 4 weeks of dithranol treatment when a uniform brown discoloration was present. Psoriatic lesions covered by thin loose scales were hyperreactive to DMSO whereas thick hyperkeratotic plaques were unreactive (3·8+ vs 0). Four weeks of dithranol treatment abolished the DMSO weal (3·6+ vs 0) on normal skin.

Correlation with primary irritants

Sodium lauryl sulphate (*SLS*). The DMSO test was performed on the ventral forearm of nineteen subjects. Five per cent SLS was applied occlusively to the opposite forearm for 24 h using aluminium chambers. The inflammatory response was evaluated 3 h after removal (1+ slight, 2+ moderate, 3+ severe). The results are shown in Fig. 7. The test panel may be divided into three groups based on the reaction to 100% DMSO. The highly sensitive Group I (3+ and 4+ reactions) also gave the strongest reactions to SLS. DMSO unreactive subjects (Group III) reacted slightly or not at all to SLS. Medium DMSO reactors (Group II) gave intermediate responses to SLS.

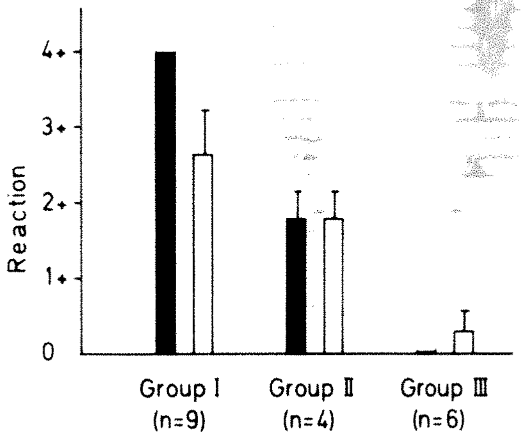

FIGURE 7. Correlation between DMSO wealing and irritant reaction to sodium lauryl sulphate. Subjects with high sensitivity to DMSO are also strongly irritated by SLS. Solid columns: DMSO weal; open columns: SLS erythema.

Ammonium hydroxide. As we have shown elsewhere (Frosch & Kligman, 1977), the minimal blistering time (MBT) to ammonium hydroxide is a sensitive indicator of barrier function; the MBT increases with the number of horny cell layers. We compared the MBT's and DMSO responses on opposite forearms of forty-eight white subjects. The results are shown in Fig. 8.

The DMSO response was inversely correlated with the MBT response. High MBT's were associated with low or negative DMSO reactions whereas low MBT's were associated with strong DMSO reactions.

DISCUSSION

We have provided data regarding the various factors that influence the cutaneous response to topically applied DMSO. The ultimate objective is to incorporate the DMSO test into a battery of procedures which can be used to assess the physiological status of skin in health and disease.

It is to be expected that various diseases affecting the horny layer barrier, the vasculature and connective tissue, will alter the weal response. It is therefore important to understand the physio-

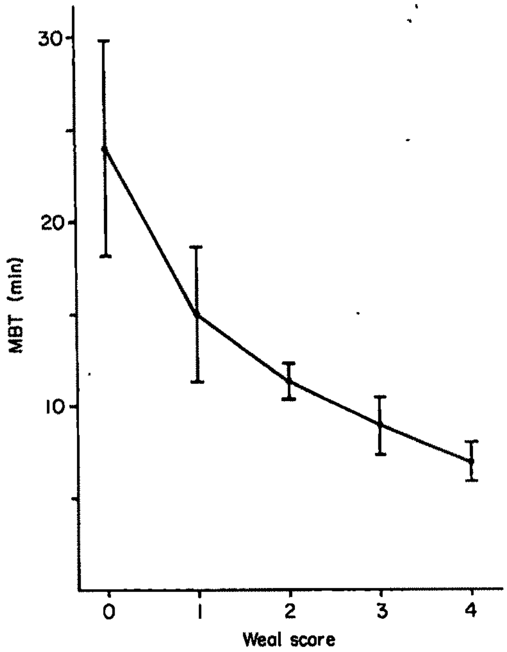

FIGURE 8. Inverse relationship between DMSO reaction and minimal blistering time (MBT) to ammonium hydroxide. High MBTs are associated with weak or negative DMSO reactions (n = 48).

logical components that determine the magnitude of the response. This must be cautiously approached even though the weal seems so simple, a mere transient engorgement of the dermis with water. Actually, a multiplicity of factors are involved. First and foremost is the rate of penetration of DMSO. This of course is dependent on the thickness and integrity of the stratum corneum. The great importance of the barrier has been illustrated several times in this study, so much so that one might be tempted to describe the DMSO procedure as a barrier function test.

Damaging the stratum corneum invariably increased the weal response, regardless of the nature of the injury, *viz.* stripping, scarification, prolonged hydration, chemical denaturation with surfactants, etc. Thickening of the horny layer by dithranol or by PUVA virtually blocked the weal (manuscript in preparation).

The profound regional variations reflect mainly differences in permeability of the barrier. The rank order of decreasing responsiveness correlates well with *in vivo* measurements of penetration such as those by Feldman & Maibach (1967) using radioactive hydrocortisone. The conspicuously high permeability of the head region is to be expected from the high density of appendageal shunts, especially the follicles. It is significant in this regard that the DMSO response begins around the follicles. The greater number of cell layers of the stratum corneum of blacks (Weigand & Gaylor, 1974) is probably the main reason for lesser wealing responses in this race.

Once having crossed the horny layer there are other factors that can influence wealing. The first is the density of mast cells which have been shown to undergo degranulation with DMSO (Kligman, 1965).

Our finding of increasing wealing responses after repeated exposures suggest that the microvasculature is progressively damaged, although damage to the horny layer is also a factor. The DMSO weal is probably due mainly to damage of the endothelium of small blood vessels with subsequent leakage of fluid. Histamine release is probably less important. Skog & Wahlberg (1967) found that high concentrations of DMSO induced intraepidermal vesiculation with perivascular infiltration of leukocytes. In one very sensitive subject we saw fine vesicles after a single 5 min application of undiluted DMSO. Montes *et al.* (1967) described profound ultrastructural changes in the horny layer of guinea-pig after a single local application of 90% DMSO.

Further evidence against attributing DMSO wealing to histamine release is afforded by the result obtained on sites previously injected with 48/80. The weal should have decreased because of mast cell degranulation. In fact, DMSO wealing increased. According to the studies of James & Eady (1979) 48/80 causes marked endothelial swelling and an extensive perivascular infiltrate of neutrophil and eosinophil leukocytes. Thus, DMSO weals tend to be enhanced whenever there has been preceding vascular damage.

Another factor is the rate of clearance of DMSO, a freely diffusable substance which can be rapidly removed after reaching the dermis. The clearance rate will depend on the rate of movement of interstitial fluid produced by the capillaries. This is equivalent to saying that the weal response reflects effective blood flow. So, increased temperature should reduce the weal and local cooling should increase it. Our findings are consistent with this prediction.

Equivalent weals in different body regions were resorbed at different rates. The head region with its richer blood supply exhibited quicker clearance.

As expected, raising a weal in adrenalin-vasoconstricted skin was difficult. Adrenergic drugs also inhibit histamine wealing (Shereff *et al.*, 1973).

There is a strong correlation between DMSO wealing and minimal blistering time (MBT) to ammonium hydroxide. With both agents, the response decreases as the number of horny cell layers increases. Thus it was scarcely surprising that subjects with shorter MBT's invariably had stronger DMSO reactions. Weals could hardly be elicited in subjects whose MBT's exceeded 20 min.

An analogous situation prevails in the relationship of wealing to irritation by sodium lauryl sulphate (SLS). Subjects who were hyperirritable to the surfactant reacted strongly to DMSO. The DMSO test may be a simpler way of identifying persons with an unusual susceptibility to toxic chemicals.

ACKNOWLEDGMENT

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The measurement of itch with sensitive limb movement meters

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SUMMARY

A sensitive electromagnetic movement detector has been developed to estimate itch by measuring scratch during sleep. The cumulative time of nocturnal limb movements of patients with itchy and non-itchy liver diseases was estimated. Non-itchy patients moved their arms for 24±9·3 min/8 h (mean±s.d.) and their legs for 16±7·0 min/8 h. The limb movements were not related to left or right handedness or the sex or age of the patient. Itchy patients had increased nocturnal limb movements; however, the increase in arm movement (up to five times control level) was much greater than the increase in leg movement (up to twice control level). The marked increase in arm movements in itchy patients supports the view that this movement is largely due to scratching. The reproducibility of the estimates of arm movements was reasonable (within-person coefficient of variation 22%). In three studies, treatment resulted in a reduction of arm movements to normal levels. The measurement of cumulative nocturnal arm movement by these sensitive meters appears to be a valid estimate of scratch and superior to a subjective assessment of itch. The devices may also be applied to other studies, such as the effects of hypnotic drugs on sleep movement and the measurement of vibration.

The investigation of itch has been hindered by the lack of an objective measurement of this sensation. Hitherto, assessment has relied on a patient's subjective impression of the symptom. An alternative approach has been to measure scratch which is the usual consequence of itch. Scratch has been assessed during sleep in itchy skin diseases using closed circuit television, action potentials from forearm electrodes and vibration transducers attached to the bed (Savin, Paterson & Oswald, 1973; Felix & Shuster, 1975; Savin *et al.*, 1975). These studies demonstrated that scratching occurs in bouts of variable duration throughout sleep. Increased severity of itch was accompanied by an increase in the frequency of the scratching bouts but their duration in an individual patient was relatively constant. Similar results were obtained from the patient when he was awake.

Instead of these complicated and expensive methods Felix & Shuster (1975) estimated scratch by measuring nocturnal limb movement with meters attached to the limbs. They demonstrated a reasonable correlation between nocturnal limb movement and both scratch estimated by vibration trans-

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ducers and the patient's subjective assessment of itch. However, our trials with these modified 'self-winding' watches revealed several disadvantages including inadequate sensitivity to record all scratch movement. In this paper we describe the use of a novel electronic meter for measuring limb movement and its value in the assessment of itch.

PATIENTS AND METHODS

Limb movement meters

Preliminary experiments showed that the range, direction and velocity of scratch movement varied widely in different patients. To record all scratch movements a sensitive meter, which was responsive in all planes, was designed. This recorded most of the movement of a particular limb. The meter (NRDC patent pending) consists of an electromagnetic movement detector whose output is amplified to actuate a retriggerable monostable which controls a mechanical or quartz watch (Fig. 1).

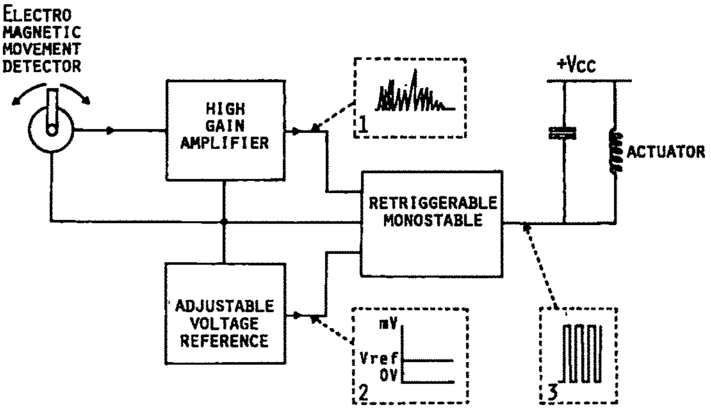

FIGURE 1. Block diagram of the movement meter circuit. The output from the electromagnetic movement detector is amplified (1) and triggers the monostable if the amplified signal exceeds the reference voltage (2). The retriggerable monostable output (3) drives the actuator controlling the watch. Cumulative error due to hysteresis is reduced to negligible proportions by the use of a retriggerable monostable running at high frequency.

Calibration is simply achieved by setting the comparator threshold voltage. This instrument provides a direct recording of the cumulative time spent moving. Before the patient goes to sleep, the movement meters, which are a little larger than a wrist watch, are attached to each wrist and ankle with a rubber strap and a 'Velcro' fastener. The meters are removed on waking. The times of attaching and removing the meters are recorded by the subject. This period is taken as the duration of sleep. Most patients performed this simple procedure unaided. The elapsed time on each meter is recorded by one of us. The observations were carried out on patients sleeping in four-bedded wards on the medical unit at the Royal Free Hospital. The sensitivity of the limb meters was assessed by measuring their response to simulated mild scratching for 1, 2 and 3 min periods in nine normal subjects. There was a direct relationship between the duration of scratching (x) and the recorded time on the limb meter (y) $(y = 1{\cdot}0; x = 2{\cdot}2; r = 0{\cdot}9995)$.

Patients

The nocturnal limb movements of fifty-three patients were recorded. Since the meters were developed to investigate itching in the cholestatic syndrome, all the subjects were in-patients with either itchy

TABLE 1. Clinical details of patients

Diagnosis	Itchy	Non-itchy	Restless
Bile duct obstruction	19	4	2
Hepatitis	0	3	0
Cirrhosis	0	5	1
Abdominal pain	0	0	3
Portal-systemic encephalopathy	0	0	2
Miscellaneous	7	7	0
Total	26	19	8

or non-itchy liver disease (Table 1). Their ages ranged between 16 and 76 years; twenty-eight were male and twenty-five female. Twenty-four of these patients wore the movement meters for 2 or more consecutive nights. In three patients the effect of percutaneous biliary drainage on scratching was studied for periods of thirteen to twenty-two consecutive nights. In eight patients, regular night sedation was continued, otherwise sedatives were withheld. The patients were classified according to whether they complained of itch. A small group of patients who had restless nights, usually due to abdominal pain or portal-systemic encephalopathy, were analysed separately.

RESULTS

The mean duration of sleep in 33 nights for non-itchy patients was 7·7±0·2 h (mean±s.e.). The duration of sleep in the itchy patients (50 nights) was slightly longer (8·3±0·2 h). The restless group (11 nights) slept for a mean of 8·2±0·5 h. To allow comparison between patients the movement meters' results were expressed per 8 h sleep. Since the movement recorded by the meters was not influenced by left or right handedness, the results were expressed as the mean of the movement of both arms and legs.

Non-itchy patients

Nineteen patients were studied for 33 nights. The movement of the arms and legs was not related to the sex or age of the patient or to the length of sleep. The mean arm movement in 8 h sleep was 24±9·3 min (mean±s.d.) and the mean leg movement 16±7.0 min. The upper limit of the normal range (mean+2 s.d.) was 43 min/8 h for the arms (Fig. 2) and 30 min/8 h for the legs.

Itchy patients

Twenty-three itchy patients were studied for 50 nights. These patients were classified, according to their assessment of the severity of the itch, into mild, moderate and severe groups. The relationship between the mean nocturnal limb movement and the itching score is shown in Fig. 3. The mean nocturnal limb movement of the non-itchy patients is shown for comparison. It is apparent that the movement of both the arms and legs increased with the severity of itching, but the increase in arm movement was much more marked. The mean time for arm movement of the severe group was about five times higher than the control level whereas the corresponding time of leg movement was only twice as high. These results imply that while itching causes some restlessness, indicated by the increase in leg movement, the much larger increase in arm movement probably represents scratching. The data for the individual patients (Fig. 2) revealed a wide range of arm movement in the itchy patients.

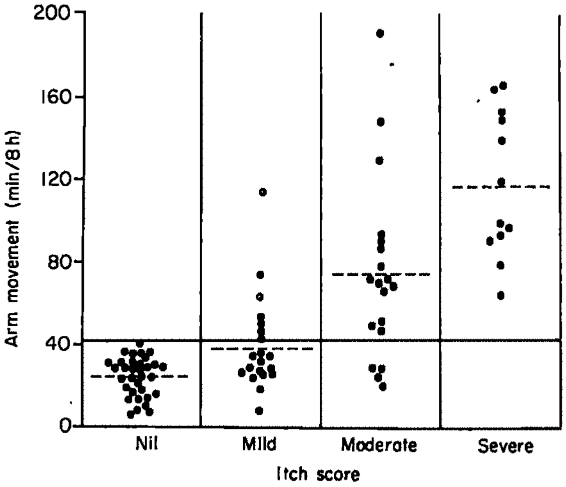

FIGURE 2. The arm movements during sleep in non-itchy and itchy patients. The solid horizontal line is the upper limit of the normal range (mean + 2 s.d.). The dotted lines represent the means of each group.

Restless patients

Eight patients, who were studied for 11 nights, complained of restless nights. The two patients with bile duct obstruction also itched. One of these patients, with hydatid cysts obstructing the bile ducts, developed pain due to the cysts on the third night of observation. The second patient with primary biliary cirrhosis had a disturbed night due to ward noise on second night of observation. These 2 nights were analysed in the restless group. The limb movements of restless patients were characteristically different from the itchy patients. Both the arm movements (73 ± 11 min/8 h; mean ± s.e.)

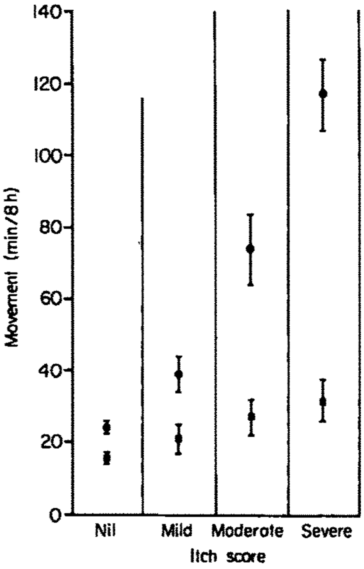

FIGURE 3. The relationship between the movements of the arms and legs during sleep in non-itchy and itchy patients (mean + s.e.). The itch score was assessed subjectively by the patient. (●) Arm; (■) leg.

and leg movements (49±13 min/8 h) were increased. In contrast to the itchy patients, restless patients had similar increases in both arm and leg movement times (approximately three times control level). The distinction between itchy and restless patients could not be improved either by subtracting leg movements from arm movements or by expressing the results as the ratio of arm to leg movement.

Reproducibility

The limb meters were worn for 10 consecutive nights by one normal subject. During 8 h sleep, arm movement (20±3·9 min) (mean±s.d.) was approximately twice as great as leg movement (9·1±1·9

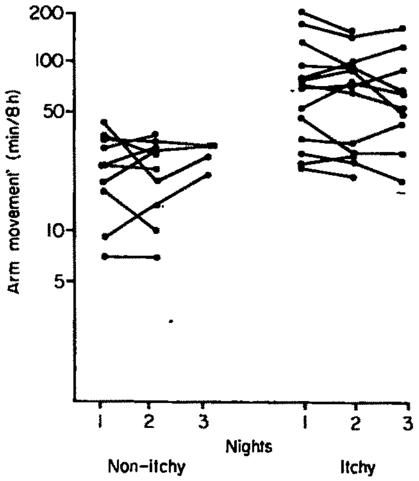

FIGURE 4. The reproducibility of arm movements during sleep in non-itchy and itchy patients studied for 2 or 3 consecutive nights. The within-person coefficient of variation for arm movement was 22%.

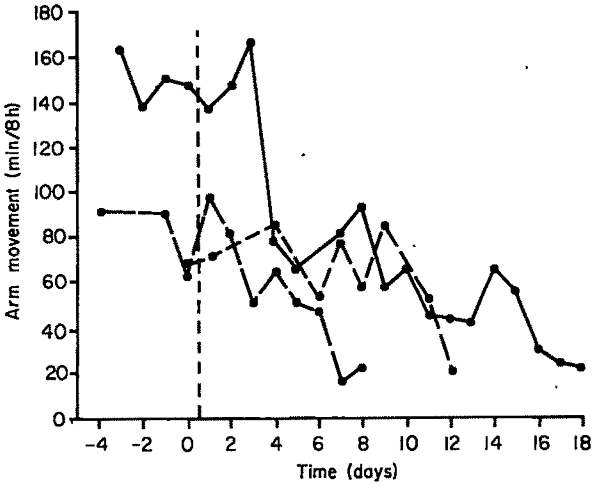

FIGURE 5. The effect of treatment (percutaneous biliary drainage) on nocturnal arm movement in three patients with severe itching due to malignant bile duct obstruction. The arm movement returned to the normal range (<43 min/8 h) between 6 and 15 days after treatment. The subjective assessment of itch tended to improve before the arm movements. Dotted line represents biliary drainage.

min). The coefficient of variation of the arm movement was 19% and of the leg movement 21%. Twenty-four patients wore the limb meters for 2 or 3 consecutive nights (Fig. 4). The variation in arm movement on consecutive nights was similar in both itchy and non-itchy patients (within person coefficient of variation 22%). Similar results were obtained for leg movement.

The effect of treatment

Three patients with severe cholestasis due to bile duct carcinoma were treated by percutaneous transhepatic biliary drainage (Fig. 5). Before treatment, the arm movement was increased in all the patients (68–163 min/8 h). Biliary drainage was followed by a reduction in arm movement. However, the rates of reduction varied; nocturnal arm movement returned to the normal range (<43 min/8 h) after 6, 11 and 15 days. It is of interest that the patients, sensation of itch tended to disappear before the arm movement had decreased to normal. Except for 3 nights, the mean leg movement in these patients was always within the normal range (<30 min/8 h).

DISCUSSION

This study has attempted to estimate the sensation of itch by measuring its motor response, which is scratch. By measuring scratch during sleep, the non-scratch movement of wakefulness should be largely eliminated. The validity of this technique depends firstly on whether similar amounts of scratch occur while asleep as when awake and secondly on a direct relationship between scratch and itch. Scratching occurs throughout sleep although the frequency of the bouts decreases with increasing depth of sleep (Savin *et al.*, 1975). The frequency of scratching bouts is similar when the patient is awake (Savin *et al.*, 1975), but the duration of these bouts is longer. This may be due to the fact that scratching is probably a reflex action during sleep and when awake there is an additional component. Furthermore, Felix & Shuster (1975) have shown that an estimate of total limb movement by movement meters is related both to direct measurements of scratch with vibration transducers and to the severity of the patients symptom of itch.

However, the modified 'self winding' watches employed by Felix & Shuster (1975) had several limitations. These included inadequate sensitivity to respond to all scratch movement and, because of the mass of the pendulum, a marked position sensitivity so that it did not respond to movement in all planes. Furthermore, the response of their system was related to the velocity of the scratch movement so that the response to vigorous scratching was greater than the response to mild scratching. In consequence, calibrating these meters on a rotating table at constant velocity was invalid. Finally, the output from the modified 'self-winding' watches is in arbitrary units, thereby making comparisons between groups difficult.

The limb meters used in this study overcome these problems and provide a record of the accumulated time spent moving. However, because of their sensitivity scratch cannot be distinguished from other movement. Arm movement in the non-itchy patients occurred in up to 9% (mean 5%) of sleeping time and the leg movement in up to 6% (mean 3%). Nocturnal limb movement was independent of left or right handedness, the patients' sex or age. Itching patients moved their arms and legs more during sleep (Fig. 3); however, increasing itchiness resulted in a much greater increase in arm movement than leg movement. In the patients with severe itching, arm movement accounted for a mean of 24% sleeping time and leg movement for a mean of 7% of sleeping time. These observations support the view that the increase in arm movement in itchy patients was largely due to scratching and are consistent with the findings of Savin *et al.* (1975) that this is responsible for most of their nocturnal arm movement. There was a wide variation in the arm movement between the itching patients (Fig. 2). This probably reflects the variability of the patients' subjective assessment of itch.

Such diverse factors as temperature (Shelley & Arthur, 1957), psychological stress and the previous experience of an itchy dermatosis (Edwards *et al.*, 1976) will alter the perception of the symptom. Nevertheless, the reproducibility of the estimates of arm movement (Fig. 4) was reasonable in the same patient (within-person coefficient of variation 22%).

This study included a small group of patients who had restless nights, usually due to pain or portal-systemic encephalopathy with a day-night reversal of their sleep pattern. We have confirmed the observations of Felix & Shuster (1975) that the limb movements of these patients are characterized by a marked increase in leg movement, but have been unable to improve the distinction between restless and itchy patients by taking account of this.

The serial studies in three patients with malignant bile duct obstruction and severe itch (Fig. 5) lends further support to the validity of this technique for estimating itch. Percutaneous biliary drainage, which reliably reduces itching, caused a fall in nocturnal arm movement in all the patients. It is noteworthy that the reduction in nocturnal arm movement usually followed an improvement in the patients' assessment of itch.

The data suggest that measurement of cumulative nocturnal arm movement by these sensitive limb meters provides a valid estimate of scratch. Since scratch is related to the patients' assessment of itch, this simple technique appears to offer an objective assessment of itch. Apart from their value in the investigation of the causes and treatment of itchy skin diseases, these movement meters may have other roles. These include the study of the effect of hypnotic drugs on limb movements during normal sleep. Since the sensitivity of the meters is easily adjusted to respond to more severe movement, events such as nocturnal fits may be recorded. Further adaptation will permit the measurement of vibration. The output from the detector can be stored in analog form to be read later on a central reader. This would dispense with the need to link the meter to a watch and make it even smaller. In conclusion, these inexpensive but sensitive movement meters appear to be superior to subjective assessment for the measurement of itch.

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IgE antibodies to house dust mite in patients with scabies

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SUMMARY

Of 135 patients with scabies, forty-seven (35%) had a positive (Classes 1–4) radioallergosorbent test (RAST) to *Dermatophagoides pteronyssinus* (DP). Elevated serum IgE concentrations were found in sixty-one (45%) of the patients. Atopic disease was confirmed in twenty-eight (23%) of the 120 patients who completed a medical questionnaire satisfactorily.

In a control group, well-matched for age and sex, of 201 employees at a local bank, only four (2%) had a positive RAST to DP, and eight (4%) had raised serum IgE levels. Atopic disease was confirmed in eighteen (9%) of them.

These observations indicate that scabies infection stimulates the production of IgE antibodies, even in non-atopic individuals, and may suggest that IgE antibodies against the human pathogenic mite *Sarcoptes scabiei* cross-react with DP. Scabies infection in addition often seems to be associated with atopic disease.

Elevated concentrations of IgE in serum are assumed to indicate either allergic disease or parasitic infection (Ishizaka, Ishizaka & Hornbrook, 1966; Johansson, Mellbin & Vahlquist, 1968) and have been reported in a proportion of patients with allergic asthma, hay fever, atopic dermatitis and urticaria (Berg & Johansson, 1969; Gurevitch *et al.*, 1973; Havnen *et al.*, 1973; Johansson & Bennich, 1967; Juhlin *et al.*, 1969).

House dust is an important allergen responsible for extrinsic asthma, conjunctivitis and rhinitis (Aas, 1971), and has been shown to contain the acarine mite *Dermatophagoides pteronyssinus* (DP) (Voorhorst *et al.*, 1967). It has also been shown that different mites of the genus Dermatophagoides have antigens in common with those found in house dust (Stenius & Wide, 1969).

Apart from asthma, house dust mites can affect man and cause fairly severe dermatoses, DP especially in Europe and *D. farinae* (DF) in America (Busvine, 1977).

One of us has previously found increased serum IgE concentrations in patients with scabies (Falk, 1980). The present study represents an attempt to elucidate the basis of skin sensitization in scabies, and concerns the relationship between scabies and antibodies to DP as well as the incidence of atopic disease in patients with scabies. We have used DP, although clearly it would have been far better to use a scabies mite antigen, but so far we have not been able to obtain sufficient scabies mite antigen.

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MATERIALS AND METHODS

Patients

One hundred and thirty-five patients (sixty-nine women and sixty-six men) between the ages of 15 and 79 years (average 27) with clinically manifest scabies were studied. In all patients diagnosis was confirmed by the presence of one or more living mites. All patients under 15 years of age were excluded, and none of the patients investigated had earlier been infected with scabies.

Two hundred and one unselected individuals among employees at a local bank served as controls. Their age (16–65 years, mean 35) and sex (ninety-seven women and 104 men) distributions did accidentally match well with those of the patient group. Also, forty-one individuals among employees at the Department of Dermatology served as controls for additional testing. Their age (22–61 years, mean 38) and sex (thirty-four women and seven men) distributions were not matched with those of the patient group.

Patients and controls were asked to answer a comprehensive systematic medical questionnaire about personal and family history of atopy. Atopic patients are here defined as patients with either current atopic disease or a past personal history of such illness, including atopic dermatitis, allergic rhinitis or extrinsic asthma. Except for the control group of 201 individuals, latent atopic patients with positive skin tests and demonstration of circulating specific IgE antibodies other than to DP were also recorded. The questionnaires were supplemented by a personal interview, the results of which were evaluated by both authors, independently.

The experiment was conducted from 1977 to 1979.

Sera

Sera, collected from the patients during scabies infection, and from the controls, were stored at −20°C until used from 6 weeks to 21 months later.

Skin tests

Direct skin tests were performed using the prick test with thirteen commercially available allergen extracts, obtained from ALK (Allergologisk Laboratorium, Copenhagen), namely *Dermatophagoides pteronyssinus* (1:100), house dust (1:20), horse dander (1:100), dog dander (1:20), cat dander (1:20), cow dander (1:20), *Cladosporium herbarum* (1:20), birch pollen (1:100), mugwort pollen (1:100), timothy pollen (1:100), skimmed cow's milk (1:20), cod (1:20), and egg white (1:100). As positive (+ + +) reference, histamine HCl 1 mg/ml was used, and 0·9% NaCl with 0·5% phenol was used as a negative (−) control. The prick tests were performed and evaluated as described by Aas & Belin (1974).

Serum IgE

The IgE concentrations in serum were determined using the Phadebas-PRIST-test (Pharmacia AB, Uppsala, Sweden). The upper limit of normal IgE is 122 u/ml in adults (Nyco, Oslo, Norway).

Radioallergosorbent test (RAST)

The Phadebas RAST test (Pharmacia AB, Uppsala, Sweden) was used to quantify circulating IgE antibodies to the same antigens as described under skin tests. Forty sera were also investigated for DF antibodies. The RAST results were expressed in Phadebas RAST Classes 0, 1, 2, 3 and 4, according to recommendations from the manufacturer and employing a reference system established by running four reference sera (A, B, C and D) with reference discs during each assay in parallel with the unknown samples. In the present study Class 1 or more was considered to be a positive RAST result, although it is possible that some Class 1 results represent non-specific reactions due to a raised IgE level.

Statistical methods

To compare two groups, the Chi-square (χ^2) test (with Yates correction) was used as described by Kemp (1942).

RESULTS

Of our 135 patients with scabies, 34·8% (47/135) had a positive RAST to DP (Table 1), and most of them belonged to RAST Classes 2–4 (Fig. 1). Only 2% (4/201) of the controls had a positive RAST to DP. This difference is highly significant ($P<0{\cdot}001$). In a second control group, 2·4% (1/41) had a positive RAST to DP. Of the forty sera investigated for IgE antibodies to both DP and DF, ten were positive to DP and eight of these ten were positive also to DF.

TABLE 1. IgE antibodies to *Dermatophagoides pteronyssinus* in patients with scabies and in a control group. The number of individuals with (RAST positive) and without such antibodies (RAST negative) are indicated, as well as the number of these with elevated total serum IgE and with a history of atopy

		Total	IgE		Atopy	
			Elevated	Normal	+	−
Patients RAST	Positive	47	41	6	16	24
	Negative	88	20	68	12	68
Controls RAST	Positive	4	3	1	4	0
	Negative	197	5	192	14	183

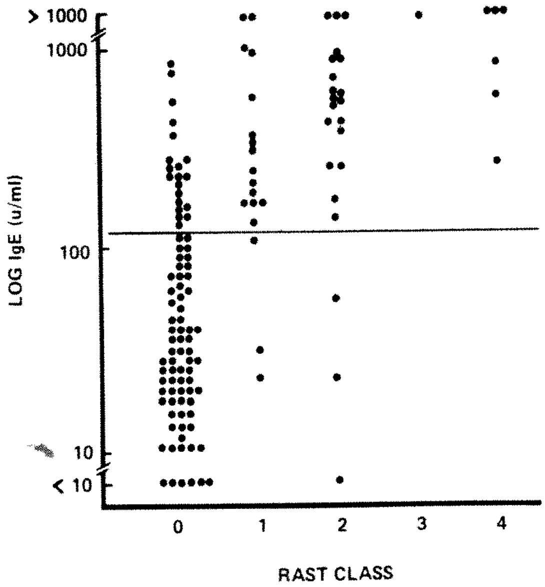

FIGURE 1. RAST results compared with total IgE values in 135 patients with scabies. Horizontal line shows upper limit of normal IgE values for adults.

Elevated IgE levels were found in 45·2% (61/135) of the patients, as opposed to only 4% (8/201) among the controls ($P<0·001$). Nearly all of the RAST-positive patients (87·2%) had elevated IgE levels as opposed to only 22·7% of the RAST negative patients ($P<0·001$) (Table 1).

Of our 120 patients with scabies who completed the questionnaires satisfactorily, 23·3% (28/120) had a personal history of atopy, as opposed to 9% (18/201) of the controls ($P<0·001$). In the second control group of forty-one employees at the Department of Dermatology, 7·3% (3/41) had a history of atopy. Among the RAST-positive patients, there were more atopic individuals (16/40) than among the RAST-negative patients (12/80) ($P<0·01$) (Table 1). The atopic group of twenty-eight patients had higher IgE levels than the non-atopic group of ninety-two patients, and there were more atopic patients among the higher RAST classes than among the lower. No statistical differences between the sexes were observed in the atopic group (twelve females and sixteen males). Interestingly, the highest RAST classes to DP were found in males (Table 2) and also the highest IgE levels. Also, atopic individuals with skin sensitivity to DP seemed to have a more severe rash than non-allergic persons. Neither age of the patients nor duration of manifest scabies infestation seemed to influence the results of the serum studies.

TABLE 2. RAST results in relation to sex and number of individuals

	RAST Class				
	4	3	2	1	0
Males	6	1	12	9	38
Females	0	0	10	9	50

Latent atopy could be demonstrated in twelve of the ninety-two patients with no personal history of atopy, based on direct skin tests (prick test) and finding specific circulating IgE antibodies other than DP. Of these twelve patients, six belonged to the RAST-positive and six to the RAST-negative group.

In the second control group of forty-one persons, four latent atopic individuals were found.

DISCUSSION

The high incidence of increased serum concentration of IgE, and of circulating IgE antibodies to DP, found in patients with scabies indicates that scabies infection stimulates the production of IgE antibodies. Since the house dust mite and the human scabies mite are arthropods of similar appearance, it is not unlikely that they or their excretions or secretions have allergens in common, especially in view of their similar nutrition. Also, different Dermatophagoides species cross-react, as we have shown for DP and DF. The two patients who failed to show cross-reactivity, with class 0 RAST to DF and Class 1 to DP, had specific antibody to DF, but insufficient to reach a Class 1 value.

We observed that our patients with skin sensitivity to DP had a more severe rash from scabies than had non-allergic persons, probably as an expression of a cross-reaction between the two mites. Also, the fact that we found many non-atopic patients with scabies who had positive RAST to DP may indicate that antibodies to *Sarcoptes scabiei* (SS) cross-react with DP, and that at least a partial identity exists between the antigens of SS and DP.

The incidence of atopy in our material is surprisingly high compared to the control group, although the reported incidence of atopic disease in the general population varies considerably, from 3 to 5% (Schwartz, 1952; 1975) to 20% (Carr, Berke & Becker, 1964). About 10–15% of a large unselected population is estimated to have atopy (Rajka, 1975). The concept of latent atopy (atopy without clinical manifestations) is well founded (Champion & Parish, 1972). However, criteria for the definition of latent atopy are in a state of flux. According to our definition the same specific antibody must be found both circulating and in the skin. As our largest control group of 201 individuals were not investigated for latent atopy, a comparison between patients with scabies and these controls has to be strictly limited to their history of atopy. A comparison of the total incidence of atopy between the patients and the small control group of forty-one employees at the Department of Dermatology would result in 33·3% (40/120) atopics among our patients as opposed to 17·1% (7/41) among the controls. Although it is our impression that atopic dermatitis is more common in the northern parts of Norway (where this study was conducted) than farther south, there is no reason to believe that the incidence of atopic disease in general should be higher in our area. According to our control groups, the frequency of atopic disease in our latitudes agrees closely with the Norwegian population in general. Thus, it is likely that there is a higher than normal incidence of atopy among our patients with scabies than among the Norwegian population in general. This could indicate that atopic immunology predisposes to scabies infection. Persons who develop dust mite sensitivity may be more likely to develop scabies, because the genetic susceptibility to become allergic in general makes one prone to become allergic to scabies. This might explain the association of scabies with atopic disease. As it seems likely that the production of IgE antibodies and of increased serum concentrations of IgE are secondary to scabies infection, it is impossible in those cases with raised IgE levels, positive RAST to DP and a history of atopy to confirm whether scabies infection or atopic disease is responsible for the increase in IgE antibody production, but probably both are involved.

We have no explanation for the fact that the highest RAST classes and also the highest IgE levels were seen in males (especially atopic males).

Further work to clarify these findings in scabies is in progress.

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Correlation of seasonal variation of 25-hydroxycalciferol with UV radiation dose

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SUMMARY

Plasma 25-hydroxycalciferol levels were measured in ten normal subjects at approximately 6-weekly intervals for 1 year. The mean annual level was 18·4 ng/ml with a seasonal variation from 14 to 26 ng/ml.

These values correlated well with serial estimates of personal UV radiation dose previously measured by polysulphone film badges.

Vitamin D status is thought to depend upon personal exposure to UV radiation in addition to dietary intake of D_2. Personal exposure to UV radiation varies enormously (Challoner *et al.*, 1976) although even outdoor workers and 'sun-worshippers' receive only a fraction of the radiation available. It would be useful to know exactly how UV radiation exposure is related to vitamin D status, but we have been unable to find any studies in which both vitamin D status and UV radiation exposure have been measured in the same subjects.

Vitamin D status is usually estimated by measuring blood levels of 25-hydroxycalciferol (25-OHD) because this metabolite has the highest serum concentration of all vitamin D metabolites. The original assay employed a competitive protein-binding assay with rachitic rat kidney cytosol as the binding protein (Haddad & Chyu, 1971). We have employed a variation of this technique which uses normal human serum as the binding protein (Ellis & Dixon, 1977) and we here report our method in full, since we have employed some minor modifications which have improved the reproducibility of the method in our hands. We have compared vitamin D status measured by this technique in normal subjects whose UV radiation exposures were known.

MATERIALS AND METHODS

Subjects

We studied ten normal volunteer subjects who were employed as office workers in the Aircraft Division of British Aerospace.

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Blood samples were taken for 25-OHD estimation at approximately 6-weekly intervals for 1 year (from January to December 1978).

Assay of 25-hydroxycalciferol

Sera (0·5 ml) were extracted twice with chloroform, methanol and water in a typical Bligh–Dyer extraction using approximately 400 c.p.m. of ^{3}H-25-OHD$_3$ (Radiochemical Centre, Amersham; specific activity = 22·4 mCi/mg) to assess recovery. The chloroform lipid extract was evaporated to dryness under nitrogen at 37°C and immediately resolubilized in 1 ml light petroleum: diethyl ether (2:1 v/v). These latter two solvents were purified according to Vogel (1962) or severe interference with binding resulted. Extracts were then chromatographed on 4 cm mini-columns of silicic acid (SIL-B-200, 60–200 mesh), the 25-OHD being eluted with 9 ml diethyl ether after washing with 5 ml of the petroleum: ether mixture. The ether was evaporated at 37°C as before and the residue immediately resolubilized in 1 ml absolute ethanol.

Assays were carried out in duplicate. They involved incubating 40 μl ethanolic ^{3}H-25-OHD$_3$ ($\sim$4000 c.p.m.) and 40 μl of sample extract in diluted human serum (typically 1:7000 with phosphate buffer, pH = 7·4, containing 0·005% Triton X-100 to ensure solubilization of 25-OHD) at 4°C. The zero point for each assay was obtained by repeating the incubation in 1 ml pure buffer. A standard curve was constructed on each day of assay by similarly incubating 40 μl aliquots of ethanolic solutions prepared from crystalline 25-OHD$_3$ (supplied by Dr N.T.Pollitt, Roche Products, Welwyn Garden City). Free and bound 25-OHD were separated by adding 1 ml of dextran coated charcoal suspension and these were mixed and centrifuged at 4°C, 1 ml of the supernatant being counted to assess binding.

Extraction and purification, as described above, typically results in 76·6% recovery (s.d. = 12·3%; n = 133).

Samples from a common serum pool gave a value of 29·1 ± 1·5 ng/ml (n = 5); the error quoted is the standard deviation which was 5·1% of the mean. The same serum pool assayed one month apart gave a value of 27·8 ± 1·1 ng/ml (n = 2). The inter-assay variation was 3·23% of the mean value. In another check of reproducibility, thirty sera were analysed on two occasions; the total variation expressed as a percentage of the mean was 9·25 ± 6·0%.

Distilled water was processed in the same way as the samples in order to evaluate the method blank. Using freshly prepared silicic acid and purified solvents there was no significant difference between the methods blank and the zero-binding standard. In routine use the lower limit of sensitivity of the assay is about 0·05 ng/tube (10% displacement relative to zero control) and this corresponds to 2·5 ng/ml at 100% recovery. However, sera of very low 25-OHD concentration can be assayed by varying the final extract concentration in ethanol. Samples from osteomalacic patients (n = 4) gave values ranging from less than 1 ng/ml to 5·5 ng/ml with a mean (±s.d.) of 3·1 ± 2·7 ng/ml.

UV radiation dosage

In a previous paper, we reported the measurement of personal exposure to UV radiation in the same individuals (Leach *et al.*, 1978). This study employed polysulphone film badges as dosimeters and individual exposures were measured for 2 weeks every 6 weeks or so between December 1975 and December 1977.

RESULTS

The sera from ten normal volunteers revealed a seasonal variation in 25-OHD levels in every case and the mean (±s.d.) values are shown in Fig. 1. The standard deviation reveals the considerable individual variation and the annual mean value was 18·4 ± 9·0 ng/ml (n = 83). A peak value of

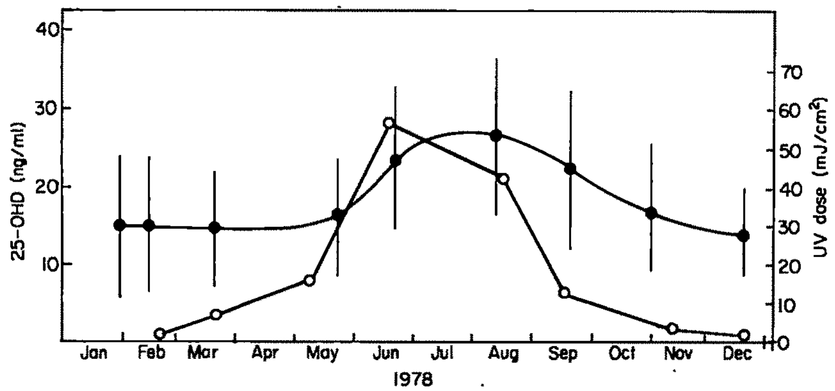

FIGURE 1. Seasonal variation of serum 25-OHD levels in Bristol office workers (●) (ng/ml ± s.d.) compared with individual exposures to UV radiation (○) (mJ/cm²).

26·3 ± 9·7 ng/ml (n = 9) occurred in August and there was a winter minimum of 14·5 ± 7·4 ng/ml (n = 10).

Unfortunately it was not possible to estimate the UV radiation dosage and the plasma 25-OHD levels in the same year, but for seven of the subjects the UV radiation exposure record for the previous 2 years was averaged, and the mean of these results were compared with the seasonal 25-OHD variation (Fig. 1).

Direct comparison of normalized values of 25-OHD and UV radiation exposure (Fig. 2) reveals a correlation which is not linear. As the UV radiation rises to a maximum value with time (see Fig. 1) then 25-OHD also rises according to the expression:

$$y = 0{\cdot}70 + 0{\cdot}25 \sin (x + 1{\cdot}25)$$

where x = normalized UV radiation exposure. Regression analysis of measured 25-OHD values and those predicted by the above expression reveals a correlation coefficient of 0·844 (n = 28) indicating

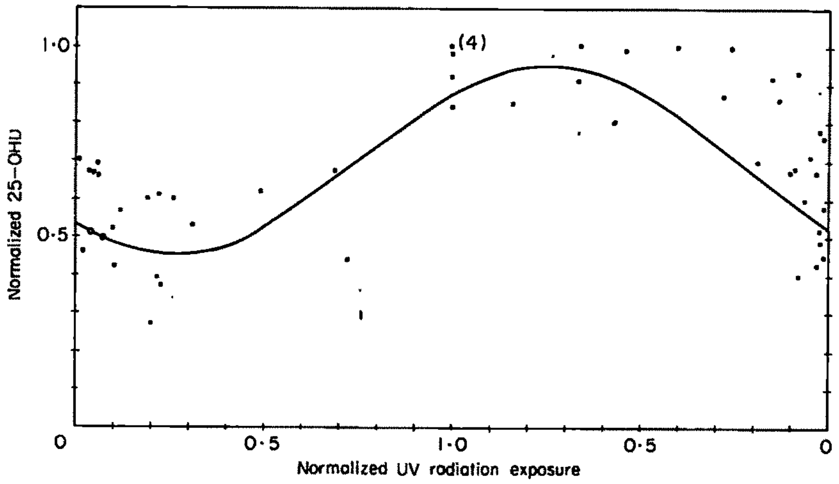

FIGURE 2. Comparison of normalized 25-OHD and UV radiation exposure values. For details of the sine curve see text.

very good agreement. A similar analysis of the decreasing 25-OHD values in the second half of the year reveals a poorer correlation coefficient of 0·73 and the 'best fit' line does not pass through the origin. Inspection of Fig. 2 reveals the poorer fit immediately; the data do in fact suggest a plateau in 25-OHD values, followed by a sharp drop when UV radiation falls below about 0·2–0·3 of its maximum value. The present data imply that maximum 25-OHD is not attained until UV radiation exposure has passed through its maximum and decreased to 0·75 that value.

DISCUSSION

The results obtained in the present study demonstrate the reliability of competitive binding protein assays in the measurement of circulating 25-OHD.

Serum 25-OHD values reported here agree well with those from other workers in the U.K. Seasonal differences between winter and summer, of 8·6 ng/ml (Belsey, DeLuca & Potts, 1976) and 17·6 ng/ml (McLaughlin *et al.*, 1974), compare well with the value of 12·4 ng/ml found here. These values are lower than the 19·3 ng/ml found by Haddad & Hahn (1973) in the U.S.A. In this study we have found winter minima of 14·5 ng/ml in 1977–78 and 13·9 ng/ml in 1978–79; McLaughlin *et al.* (1974) reported a mean value of 18·6 ng/ml in December 1972 but other winter minima have been slightly below our value (Stamp & Round, 1974; Preece *et al.*, 1974; Ellis & Dixon, 1977) in the U.K. Previously reported summer maxima in the U.K. range from 22·6 ng/ml by Ellis & Dixon (1977) to 36·2 ng/ml by McLaughlin *et al.* (1974) compared with the value of 26·3 ng/ml found here. Thus with the exception of the relatively high values of McLaughlin *et al.* (1974), the U.K. values agree reasonably well and differ markedly from the results for Switzerland (Garcia-Pascual *et al.*, 1976) and Germany (Offerman, von Herrath & Schaeffer, 1974). This serves to emphasize that, in considering the significance of abnormal 25-OHD, the 'seasonal norm' for the locale must be borne in mind.

The peak value of UV radiation in our study did not in general coincide with the peak serum 25-OHD value. The UV radiation values we used, however, were averages from different years and actual exposure of the subject depends to some extent on weather variables other than sunshine (e.g. temperature and wind strength). Our data cannot therefore be used to provide an accurate estimate of the time lag between UV radiation exposure and peak of 25-OHD production, but they do serve to emphasize the importance of personal exposure to UV radiation in producing 25-OHD.

The comparison shown in Fig. 2 reveals that the relationship between UV radiation exposure and 25-OHD differs in the increasing and decreasing phases. As UV radiation increases the increase in 25-OHD corresponds very well to a sine curve. Production of the vitamin is therefore much greater than the sum of metabolism and storage. Once UV passes its maximum, but before it drops below about 0·2–0·3 of its maximum value, 25-OHD appears to decrease so slowly as to be virtually stationary. This may imply that production and recovery from tissue is balancing metabolic use of the 25-OHD. After normalized UV radiation falls below 0·2–0·3, the 25-OHD falls rapidly, indicating that metabolic use is greater than production and recovery from tissue.

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Sister chromatid exchange and chromosome aberration rates in a group of psoriatics before and after a course of PUVA treatment

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SUMMARY

Blood was cultured and chromosome preparations were examined for sister chromatid exchanges and chromosome aberrations in eighteen patients receiving photochemotherapy with 8-MOP and UV-A for the treatment of psoriasis, both before starting treatment and again 6 months later. The UV-A was administered both by means of low output (irradiance: 1·1–2·25 mW/cm^2) and high output (irradiance: 6·1–7·6 mW/cm^2) UV-A radiation cubicles. Irrespective of the intensity of irradiation or the total dose of UV-A administered there was no evidence of chromosome damage in lymphocytes following treatment.

Sister chromatid exchanges (SCEs) may be demonstrated in chromosome preparations, following the incorporation of bromodeoxyuridine (BrdU) into the DNA in place of thymidine over two cycles of replication, by methods which differentially stain the two chromatids of a chromosome and thus reveal exchanges which occur between them (Latt, 1973; Perry & Wolff, 1974). SCE frequency may be a useful indicator of the exposure of cells to mutagens and carcinogens *in vitro* (Perry & Evans, 1975). Damage induced in human lymphocytes by 8-methoxypsoralen (8-MOP) and long-wave ultraviolet radiation (UV-A), even prior to the addition of BrdU, results in an increase in SCEs in cultures harvested at 72 h (Carter, Wolff & Schnedl, 1976; Mourelatos, Faed & Johnson, 1977a), and recently Latt & Loveday (1978), using Chinese hamster ovary cells, have demonstrated that a single dose of treatment has an effect on SCE levels which persists over multiple generations in culture. It would therefore seem reasonable to expect that if significant damage to lymphocytes occurs in patients receiving photochemotherapy involving the administration of 8-MOP followed by exposure to UV-A (PUVA) this should result in a rise in SCE levels in cells cultured for 72 h in the presence of BrdU.

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We have previously examined blood from patients suffering from psoriasis who had not been treated with photochemotherapy and from those currently receiving this form of treatment, and were unable to demonstrate any difference in SCE level (Mourelatos *et al.*, 1977b); neither did we find that this level was affected by the number of treatments received (Faed & Mourelatos, 1978a). The treatment regime, however, made use of low doses of UV-A on any one occasion and most subjects received only relatively small total doses of irradiation (Lakshmipathi *et al.*, 1977). It is possible, furthermore, that an increase in SCE frequency could have been masked by differences in the intrinsic SCE levels between patients.

In view of this possibility that chromosome damage might still occur as a result of a continuous period of psoralen photochemotherapy, we have extended our investigations of patients being treated in the Department of Dermatology in Dundee.

We now report SCE and chromosome aberration studies on eighteen patients who were examined prior to any photochemotherapy and again 6 months after starting treatment in low output UV-A cubicles (irradiance range: 1·1–2·25 mW/cm^2), or a high output cubicle (irradiance range: 6·1–7·6 mW/cm^2), or a combination of both (see Table 2).

MATERIALS AND METHODS

The technique of photochemotherapy used (Lakshmipathi *et al.*, 1977) consisted of the oral administration of 8-MOP in a dose based on the age and weight of the subject and taken two hours prior to exposure of the skin to long-wave UV radiation (UV-A 320–400 nm). Three irradiation cubicles were used, each producing a different amount of energy over a maximum period of 20 min (cubicle No. 2: 2·5 J/cm^2; cubicle No. 3: 8·0 J/cm^2; cubicle No. 4: 3·0 J/cm^2). There was some variation in output as shown in Table 1. The arbitrary maximum of 20 min exposure was chosen for reasons other than the present study. Reference to Table 2 shows that some subjects who failed to respond to the initial dosage regime were then changed to a different numbered cubicle, the higher output thereby allowing a greater exposure dose in the 20 min maximum period.

TABLE 1. Typical range of doses (J/cm^2) for each cubicle within the maximum of 20 min. There is an obvious fall off in irradiance in cubicles 2 and 4

Dose	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
Cubicle 2	1·5	1·5	1·5	2·0	2·0	2·0	2·5	1·4	1·4	1·4	1·4	1·4
Cubicle 3	5·0	5·5	5·5	6·0	6·5	6·5	6·5	6·5	6·5	7·0	7·5	8·0
Cubicle 4	1·6	2·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0	2·5	2·5	2·5

Blood was obtained from eighteen subjects prior to starting photochemotherapy and a second sample taken 6 months later. The amount of photochemotherapy received varied due to the differing clinical responses to treatment within the group of subjects studied. In approximately half those studied the second sample of blood was not taken until some time after photochemotherapy had been stopped. In the meantime, however, no treatment was given other than the topical application of vaseline if required.

The blood samples were collected into light-shielded Lithium Heparin tubes and for each culture 0·8 ml of whole blood was added to 8 ml culture medium (85% Eagle's medium, 15% calf serum, 100 unit/ml penicillin and 10^{-4} mg/ml streptomycin) in a universal container. The cultures were then injected with 12 μm BrdU and 0·1 ml phytohaemagglutinin and incubated for 72 h at 38°C

TABLE 2. UV-A treatment regime followed by each of the eighteen patients. In those patients in whom an increase in UV radiation was required the change is indicated by 4→3. The output over 20 min for each cubicle is as follows: Cubicle No. 2: 2·5 J/cm^2; Cubicle No. 3: 8·0 J/cm^2; Cubicle No. 4: 3·0 J/cm^2

No.	Name	Sex	Age	Treatment cubicle number	Duration of treatment (days)	Total dose of UV-A received prior to Sample 2 (J/cm^2)	Time interval between end of treatment and Sample 2 (days)
1	T.M.	F	22	3	182	274	No interval
2	T.D.	M	40	4	114	46	68
3	P.H.	F	26	4→3	154	213	28
4	J.G.	M	46	3	182	230	No interval
5	W.S.	M	45	4→3	182	137	No interval
6	G.R.	M	35	4→3	152	195	30
7	J.O.	M	31	4→3	182	235	No interval
8	J.W.	F	23	4	78	44	104
9	M.M.	F	35	2→3	178	222	4
10	M.G.	F	51	2→3	182	239	No interval
11	R.M.	M	51	2	88	28	94
12	A.W.	M	48	3	37	49	145
13	V.B.	M	29	4	182	69	No interval
14	G.R.	M	28	4→3	182	284	No interval
15	T.A.	M	31	2→3	182	235	No interval
16	J.S.	M	65	2	141	47	41
17	L.S.	M	35	2	182	41	No interval
18	C.F.	F	42	4→3	154	147	28

inside a light-tight box. Colchicine (0·002% w/v) was added for the last 2·5 h of culture life and chromosome preparations made by the method described by Perry & Wolff (1974), with minor modifications.

Chromosome aberrations were separately scored on cells in their first *in vitro* division (no differential staining of sister chromatids) and in their second division (all chromosomes differentially stained). Chromosome aberrations were scored separately from chromatid aberrations. SCEs were scored on cells in their second *in vitro* division. All scoring was done without knowledge of the treatment received by the patient.

RESULTS AND DISCUSSION

SCE rates before and after photochemotherapy are shown in Table 3. Although the mean SCE rate per cell ranged from 3·86 to 10·55 overall the mean difference between cultures from the same patients before and after treatment was only 1·2 exchanges (range 0·01–4·22) or 0·94 (range 0·01–2·3) if Case 14 is excluded. Case 14 is so far outside the distribution of the others that special considerations may apply although it should be noted that this individual received the highest total dose and the majority of his irradiation was received in the high output cubicle. Apart from the findings in this one individual there is no evidence from this study of a change in SCE rate as a result of photochemotherapy; there is no significant alteration in SCE rates irrespective of the manner in which the PUVA treatment was delivered.

TABLE 3. Radiation exposure, mean SCE levels before and after irradiation and aberration levels after treatment for eighteen psoriatics undergoing photochemotherapy in a 6-month period. All the high intensity irradiation was given in Cubicle 3 (see text)

	UV-A dose (J/cm^2)			Mean SCE/cell (No. of cells)		Aberrations after treatment (No. of cells examined)		
Patient	Low intensity (Cubicle No.)	High intensity	Total UV-A exposure	Before treatment	After treatment	First Division	Second Division	Total
1	0	274	274	5·33 (6)	5·07 (7)	0 (26)	0 (44)	0 (70)
2	46 (4)	0	46	6·67 (30)	4·36 (29)	1 (28)	0 (72)	1 (100)
3	61 (4)	152	213	7·83 (30)	6·14 (30)	0 (56)	1 (44)	1 (100)
4	0	230	230	7·50 (30)	5·67 (30)	1 (35)	0 (48)	1 (83)
5	113 (4)	24	137	8·70 (30)	8·47 (30)	0 (29)	3 (70)	3 (99)
6	65 (4)	130	195	7·83 (30)	7·84 (30)	0 (13)	1 (87)	1 (100)
7	65 (4)	170	235	5·73 (11)	5·60 (30)	0 (13)	1 (53)	1 (66)
8	44 (4)	0	44	6·67 (30)	6·77 (30)	1 (37)	0 (63)	1 (100)
9	32 (2)	190	222	6·22 (23)	7·73 (30)	1 (41)	3 (59)	4 (100)
10	31 (2)	208	239	5·67 (30)	5·20 (10)	0 (27)	1 (28)	1 (55)
11	28 (2)	0	28	5·25 (12)	5·50 (2)	2 (13)	0 (2)	2 (15)
12	0	49	49	6·17 (30)	5·93 (30)	0 (40)	0 (60)	0 (100)
13	53 (4)	0	69*	4·67 (6)	4·10 (18)	0 (41)	0 (59)	0 (100)
14	65 (4)	219	284	6·33 (30)	10·55 (21)	0 (5)	0 (21)	0 (26)
15	28 (2)	207	235	5·63 (30)	7·23 (30)	1 (41)	1 (62)	2 (103)
16	47 (2)	0	47	4·07 (30)	5·90 (30)	0 (24)	1 (76)	1 (100)
17	41 (2)	0	41	5·50 (2)	3·86 (7)	—	0 (7)	0 (7)
18	54 (4)	93	147	5·61 (24)	6·91 (12)	0 (57)	0 (26)	0 (83)
Totals			2735	— (414)	— (406)	7 (526)	12 (881)	19 (1407)
Means for group			152	6·44 —	6·52 —	1·3%	1·4%	1·35%

* Includes 16 J/cm^2 over 4 weeks in a low intensity cubicle at another hospital.

Comparable results showing no increase in SCEs following photochemotherapy have been obtained by Lambert *et al.* (1978) who examined the same patients before and after treatment on a regime in which doses of about 3·0 J/cm^2 were given intensively 5 days a week, and by Wolff-Schreiner *et al.* (1977) who compared SCE levels in nineteen patients having had varying durations of treatments in which the UV-A exposure was given in individual doses varying between 0·5 and 8·0 J/cm^2 with five patients receiving total cumulative doses in excess of 800 J/cm^2.

There should, however, be some caution in interpreting these results as it has recently been suggested that B cells have lower SCE rates than T cells at equivalent BrdU concentrations and also that the majority of those B cells present at 72 h in PHA stimulated cultures from normal individuals have already reached their third *in vitro* division (Santesson, Lindahl-Kiessling & Mattsson, 1979). The effect of photochemotherapy on B cell cycling time is not known but the proportion of B cells is reported to be increased in the blood of patients on treatment (Ortonne *et al.*, 1977). Photochemotherapy may therefore have a complex effect on SCE level; in particular an increase in the proportion of B cells scored is likely to decrease SCEs scored which may therefore mask increases resulting from damage to DNA.

The nature of this damage which results in SCEs is not fully understood but it may well be, as we have argued elsewhere (Faed & Mourelatos, 1978b), that excision repair can remove lesions which would otherwise give rise to SCEs. SCEs may not therefore be particularly good indicators of chronic, low-level exposure to a damaging agent. However, we have not been able to find any evidence of a raised frequency of unrepairable damage as shown by chromosome aberrations either (Table 3) which is in agreement with other studies on aberration rates in lymphocytes of patients receiving PUVA (Swanbeck *et al.*, 1975; Brogger, Waksvik & Thune, 1978). Because of the low frequency of all kinds of aberration these have not been shown separately in Table 3. The total aberration yield was 1·3% in first division cells and 1·4% in second division cells following treatment. There is thus no evidence from this study of cumulative chromosome damage of any kind in lymphocytes of psoriatic patients being treated by PUVA.

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Acrokeratosis paraneoplastica—a new cutaneous marker of malignancy

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SUMMARY

The historical background and the main clinical features of a specific paraneoplastic dermatosis, acrokeratosis paraneoplastica, are reviewed. Typical features are erythematous scaly lesions on the extremities, ears and bridge of the nose, associated with a malignancy, most frequently in the laryngopharyngeal region.

The condition described in 1965 by Bazex and his colleagues as a skin marker of malignancy has become known as acrokeratosis paraneoplastica, or Bazex syndrome. It has been recognized with increasing frequency in France, but not so far in England. A case was presented to the Anglo-French reunion of the British Association of Dermatologists in Nancy, 1978. Dr Ian Sneddon commented that he had looked for, and failed to find, a case since the condition was described to English dermatologists at the Anglo-French meeting in Toulouse, 1970. It was felt that the condition might be recognized here if it were more widely known among English dermatologists.

We therefore present a review of the history and main features of the disorder.

REVIEW

The bibliography includes only those references which are reasonably accessible to English readers. Further material in the form of congress proceedings and theses are listed in a recent German review (Bazex, 1979).

The first description was of a patient presented to the French dermatological Society in 1965 with scaly erythematous lesions of the extremities who had a carcinoma of the pyriform fossa (Bazex *et al.*, 1965). Treatment of the carcinoma led to clearing of the skin lesions. Since 1965 further case reports and reviews appeared in the continental literature bringing the number of recorded cases to nearly forty (Bazex *et al.*, 1965, 1968, 1969; Bazex, Dupré & Christol, 1971; Bureau *et al.*, 1971; Thiers *et al.*, 1973; Bazex, 1979). All but two of these patients were French (Braverman, 1970;Cabre *et al.*, 1975). The condition has been described only in association with a malignancy.

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Since metastases from the original tumour are not found in the skin and since in the majority of reports the eruption cleared after successful treatment of the malignancy the condition was regarded as a true cutaneous marker of malignancy and was called 'acrokeratosis paraneoplastica' (APN).

The main points emerging from the case reports are:

(1) The condition affects males exclusively,* nearly always over the age of 40. So far it has been detected only in Caucasians.

(2) It appears to be associated only with neoplasia of the pharyngo-laryngeal region, oesophagus, tongue, lower lip, upper third of the lungs, or with neoplasia metastasizing to the cervical region.

(3) The initial lesions start on the extremities and affect particularly the fingers, toes, nose and ears. They are psoriasiform and usually symmetrical.

(4) Association with other cutaneous markers of malignancy has been recorded only twice (Moulin, Valignot & Bouchet, 1975; Dupré *et al.*, 1976), both patients having acquired ichthyosis.

CLINICAL DESCRIPTION

Typically three stages can be recognized.

First stage

The eruption starts as erythema and psoriasiform scaling on the fingers and toes, soon spreading to the margins of the aural helices (Figs 1 and 2), and as violaceous erythema and pityriasiform scaling over the bridge of the nose. The nail folds are prominently involved and may be tender. Elsewhere the eruption is symptomless. Nail changes are frequent and consist of dystrophy with subungual hyperkeratoses and onycholysis progressing to complete destruction of the nail plate. At this stage the neoplasm is frequently undetected but may have already metastasized to the cervical glands.

Second stage

The eruption spreads from the nail folds to the whole of the hands and feet producing keratoderma with a violaceous colour. The skin appears oedematous and the palms may have a honeycomb appearance. Red scaly lesions involve the whole of the pinna (Fig. 3), and on the face the eruption spreads from the bridge of the nose sometimes reaching the upper lip (Fig. 4). The neoplasm at this stage often gives rise to localizing signs and systemic symptoms.

Third stage

If the neoplasm is not treated the rash extends locally or new lesions appear in other sites such as the legs, knees, thighs and arms. On the trunk the lesions resemble psoriasis but the plaques are always ill-defined, and never progress to erythroderma. On the face, the lesions mimic seborrhoeic eczema or lupus erythematosus, and on the scalp the scaling is pityriasiform. In some cases the extent of the rash does not correlate with the extension of the malignancy. With successful treatment of the tumour the rash usually clears, but changes in the nails frequently persist. Atypical features have been noted in some reports, such as pruritus (Blum *et al.*, 1970), vesiculation (Degos *et al.*, 1968; Labouche *et al.*, 1973; Moulin *et al.*, 1975; Bourgeois-Spinasse, Briche & Grupper, 1976), sterile paronychia (Bureau *et al.*, 1976), hyper- and hypopigmentation (Duperrat *et al.*, 1974). The pigmentary change, although unusual, suggests a possible link with malignant acanthosis nigricans. Schnitzler *et al.* (1973) reported some improvement with antibiotics suggesting secondary bacterial infection. Incomplete forms have

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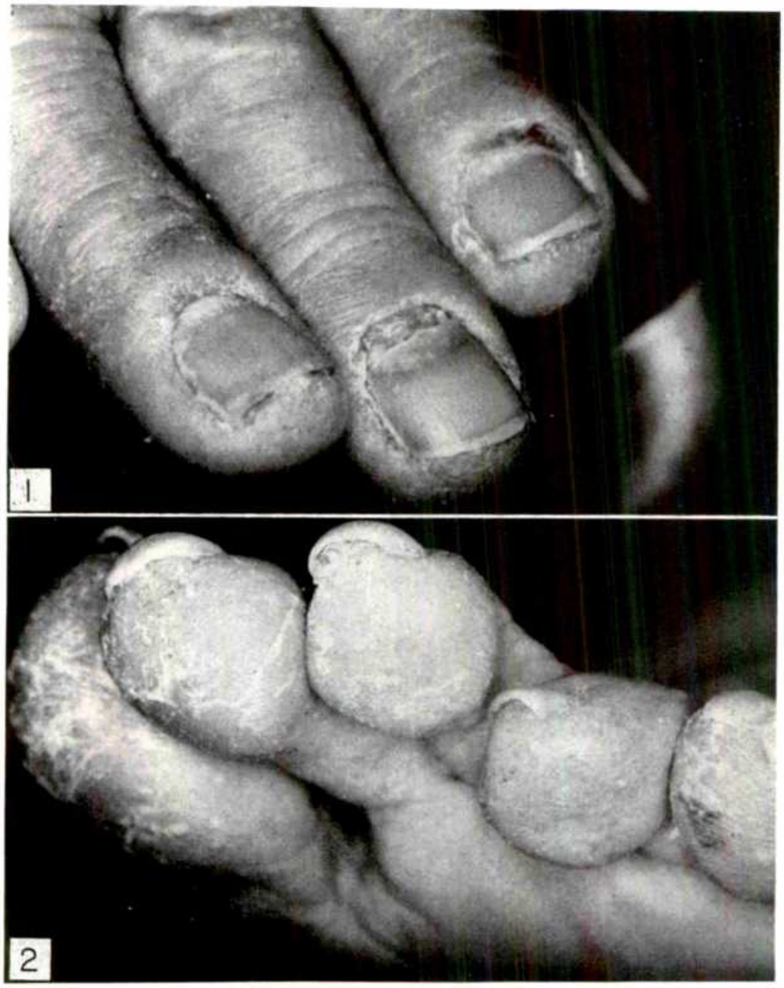
* Since submission of this paper a female case has been presented by Professor J.Civatte (1980) Acrokératose para-néoplasique: syndrome paranéoplasique d'un cancer épidermoide des bronches. *Réunion de la Société Française de Dermatologie,* held in Paris 13–15 March.



FIGURES 1 AND 2. Acrokeratosis paraneoplastica. Male aged 55. Typical lesions on fingers and toes which led to the discovery of a laryngeal carcinoma. Successful treatment of the tumour was followed by rapid resolution of the rash.

been described in which the resemblance to innocent dermatoses was misleading. The widespread lesions and the absence of a family history suggest that APN is not a variant of the syndrome associating keratoderma with carcinoma of the oesophagus.

HISTOPATHOLOGY

The tumour is usually a squamous carcinoma, occasionally undifferentiated and rarely derives from glandular cell types. The skin lesions show an ill-defined lymphocytic infiltrate around the upper dermal vessels which does not reach the dermo–epidermal junction. There are mild acanthosis and hyperkeratosis with scattered parakeratotic foci. In some cases fibrinoid degeneration in the superficial capillaries is seen (Huriez *et al.*, 1967; Bureau *et al.*, 1971). The infiltrate usually contains a few

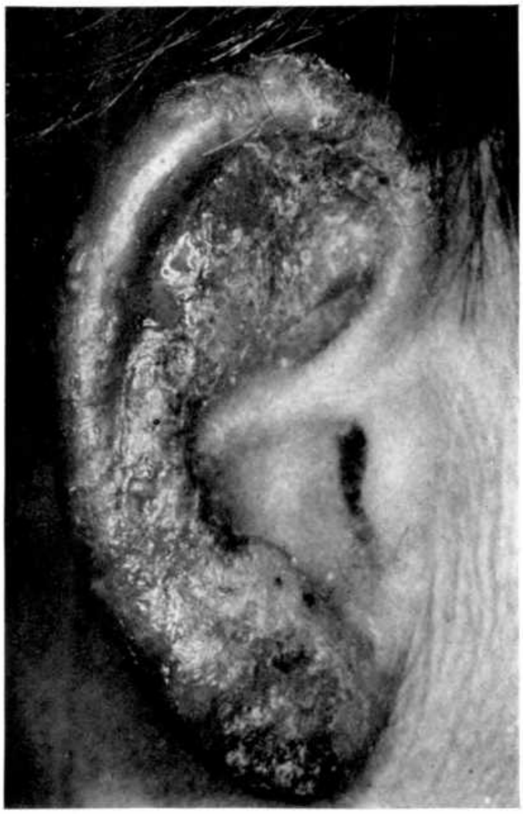


FIGURE 3. Typical lesions on ear in same case as in Figs 1 and 2.

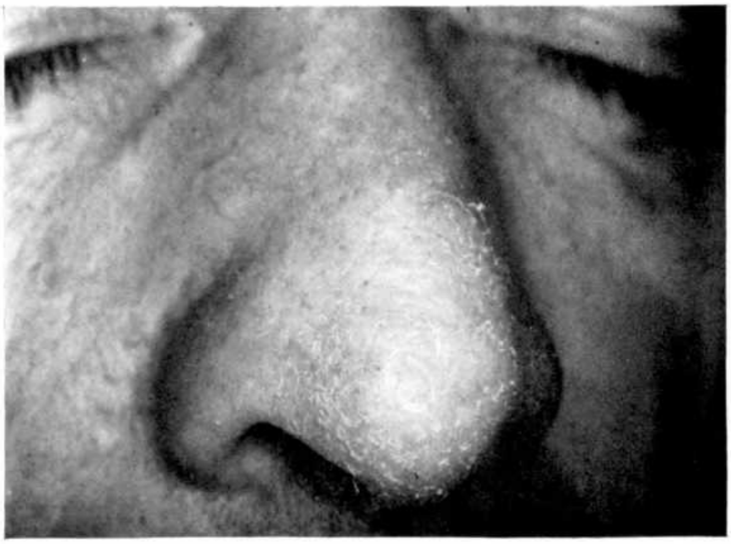


FIGURE 4. Lesions on nose of same case.

pyknotic neutrophils resembling allergic vasculitis. Other changes reported include eosinophilic hyalinisation of individual prickle cells and scattered vacuolar degeneration.

In one patient immunofluorescence showed a uniform deposit of IgG in the basal layer but this was not confirmed in another patient. Although the histopathological changes are non-specific, they allow the exclusion of psoriasis, lupus erythematosus and other similar eruptions. In several patients a large range of laboratory investigations were performed with negative results. In a thesis quoted by Bazex (1979), Bilbeissi reported a low serum vitamin A, raised gamma globulins and raised serum catecholamines.

TREATMENT

Removal of the tumour usually causes regression of the eruption. The rash reappears if the tumour relapses at the primary site or spreads to the cervical and mediastinal glands but not if it metastasizes to other sites. Symptomatic treatment with antibiotics, topical steroids or keratolytics produces only slight and transient improvement, and this lack of response is a feature supporting the diagnosis of APN.

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Scanning electron microscope studies of solar elastosis

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SUMMARY

Scanning electron microscope findings of solar elastosis were compared with those of normal dermis in specimens digested by hyaluronidase and by autoclaving. Solar elastosis showed amorphous masses and broad fibres with irregular shapes and sizes in specimens digested by hyaluronidase, while it showed only fibrous structures in those after autoclaving. It is suggested that the origin of solar elastosis is elastic tissue.

Many studies have been carried out on the structure and organization of solar elastosis by light (LM) and transmission electron microscopy (TEM) (Felsher, 1961; Banfield & Brindley, 1963; Niebauer & Stockinger, 1965; Mitchell, 1967; Ebner, 1969; Danielsen & Kobayashi, 1972; Stevanovic, 1976; Nürnberger *et al.*, 1978) although there is only one paper describing SEM findings of solar elastosis (Dawber & Shuster, 1971).

The main reason for this may be that the dermis consists of various components, the great bulk of which is collagen, and that it is hard or impossible to visualize only the elastic fibres. In addition, the ground substance makes it difficult to see fibrous structures of the dermis.

We have recently developed a method (Tsuji, Lavker & Kligman, 1979) which is based on the fact that when connective tissue is autoclaved or boiled, collagen and ground substance are removed, leaving elastic fibres in their native state.

The purpose of this report is to describe the SEM findings in solar elastosis using this new method.

MATERIALS AND METHODS

Specimens were taken from the neck and abdomen of four patients (62–80 years old) with cutis rhomboidalis nuchae. The subcutaneous tissue was cut away and the specimens were incubated in 2N sodium bromide at 37°C for 30 min. The epidermis was then gently lifted off. Approximately 1 mm thick sections of dermis were cut and processed as follows: (1) some sections were placed in 0·05% hyaluronidase solution in 0·1 mol/l phosphate buffer (pH 5·3) and control sections were put into 0·1 mol/l phosphate buffer solution (pH 5·3). (2) Some sections were placed in distilled water

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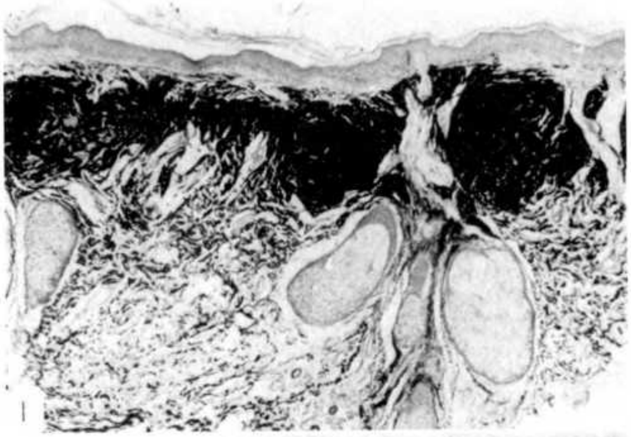


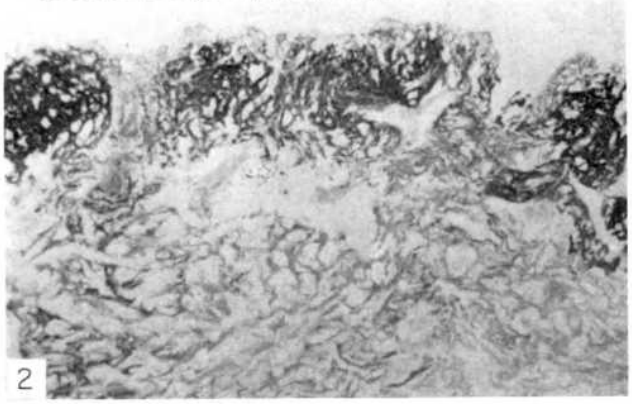


FIGURE 1. Light micrograph of a specimen from the neck prior to autoclaving or hyaluronidase digestion. Elastotic fibres are seen in the upper dermis (Weigert stain, × 80). FIGURE 2. Light micrograph of a specimen from the neck after autoclaving, showing a pure preparation of elastotic fibres whose orientation is similar to that observed in the dermis before autoclaving (Weigert stain, × 80).

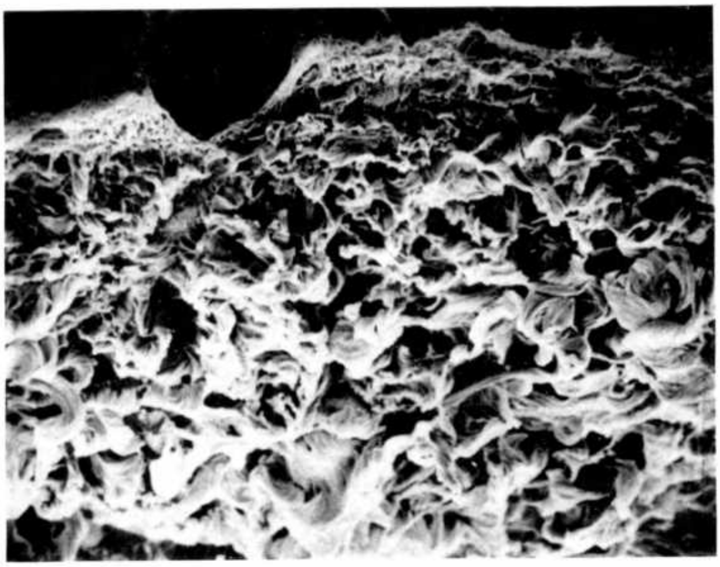
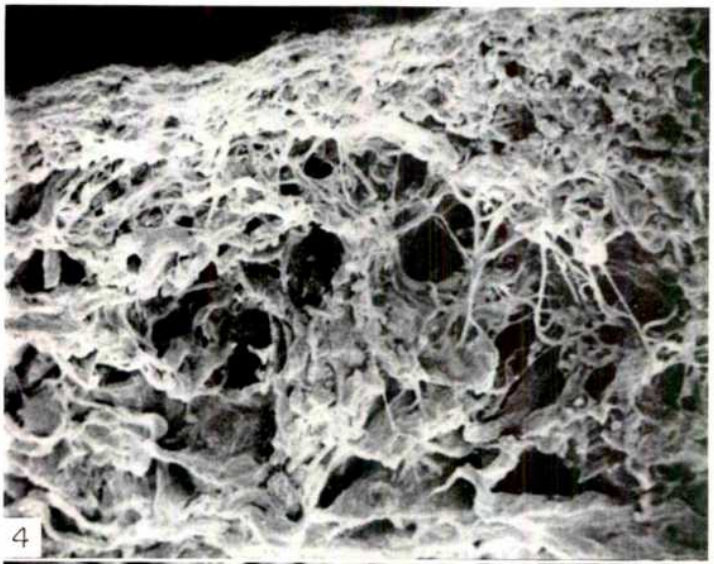


FIGURE 3. Scanning electron micrograph of a specimen from the abdomen after digestion by hyaluronidase. Collagen fibres of similar size are running horizontally to the skin surface (top of the figure). Few elastic fibres can be seen among the collagen fibres (× 400).

and autoclaved for 8 h at 121 °C at 18 psi. These sections were processed for light and scanning electron microscopy.

Light microscopy

After fixation in neutral buffered formalin, paraffin sections were stained with Weigert's stain to confirm that autoclaved specimens contain only Weigert positive fibres.



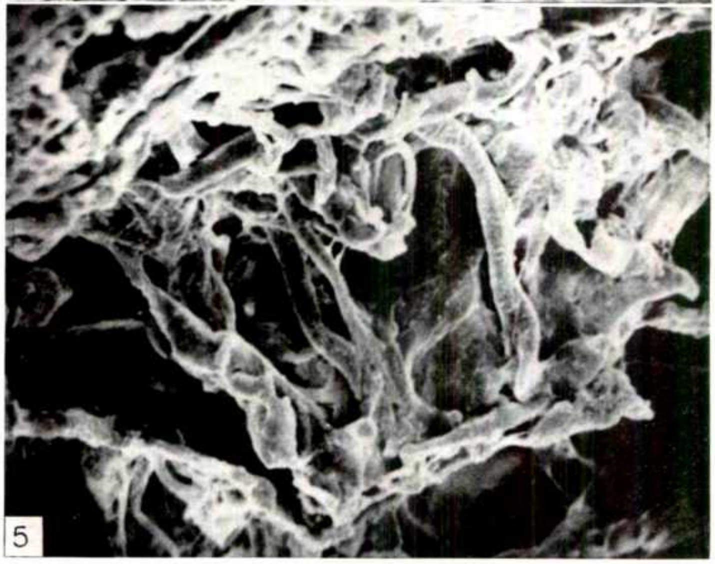


FIGURE 4. Scanning electron micrograph of a specimen from the neck after digestion by hyaluronidase. Amorphous masses and broad fibres with irregular shapes and sizes are seen in the upper dermis. Many thin, fibrous structures connect one amorphous mass to another, leaving holes between fibres and masses (× 400).

FIGURE 5. Higher magnification of a portion of Fig. 4, showing amorphous masses and broad fibres which connect with each other (× 800).

Scanning electron microscopy

The sections were fixed at room temperature in 2% paraformaldehyde-2·5% glutaraldehyde in 0·1 mol/l cacodylate-HCl buffered at pH 7·4 (Karnovsky, 1965) for 1 h, and then rinsed in buffer for 1 h. After dehydration in a graded (70–100%) series of ethanol baths, the samples were placed in 100% xylol for 2 h, and then air dried. Dried samples were mounted on aluminium stubs using silver paint. They were coated with a thin layer of gold palladium in an ISI sputter coater and viewed in a TV MINI-SEM operated at 10 kV.

RESULTS

Light microscopy

A comparison of Weigert stained elastotic fibres in histological specimens of solar elastosis before and after autoclaving revealed a similarity in organization. In both, elastotic fibres are seen in the upper dermis (Figs 1 and 2).

Scanning electron microscopy

In general, control specimens showed no clear fibrous structures in the dermis. However, after digestion by hyaluronidase they could be revealed. In specimens from the abdomen, collagen fibres of similar size were seen, running horizontally to the skin surface (Fig. 3). Few elastic fibres could be seen among the collagen fibres. In specimens from the neck, there were amorphous masses or broad fibres with irregular shapes and sizes in the upper dermis (Fig. 4). Many thin, fibrous structures connected one amorphous mass to another, leaving holes between fibres and masses with the appearance of a web (Figs 4 and 5).

After autoclaving, collagen fibres and ground substance were removed. In specimens from the abdomen, all the fibres that remained were elastic fibres. Large numbers of interwoven, long, coarsing, branched elastic fibres separated by spaces were seen (Fig. 6). They were aligned primarily horizontally

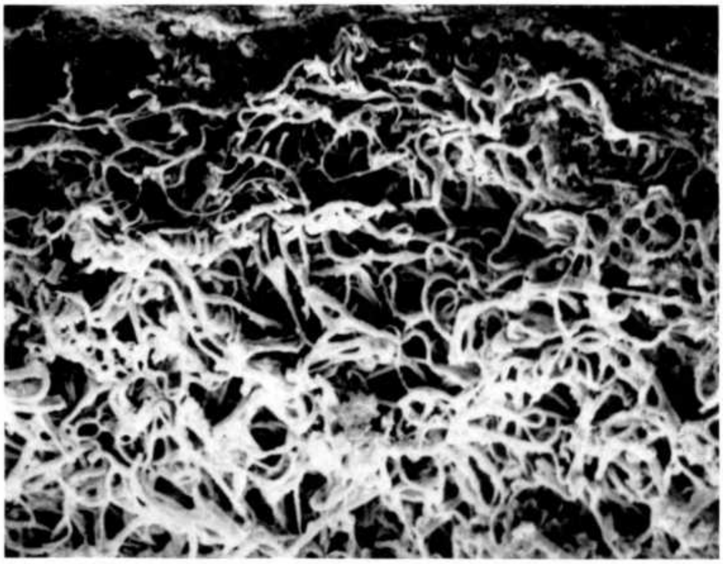
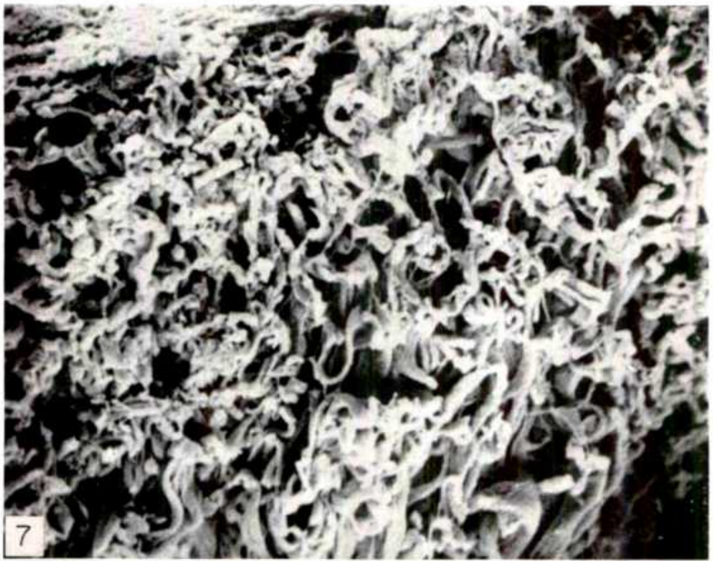


FIGURE 6. Scanning electron micrograph of a specimen from the abdomen after autoclaving. Large amounts of interwoven, long, coarsing, branched elastic fibre separated by spaces are seen. They are aligned primarily horizontally to the surface (top of the figure) (× 400).



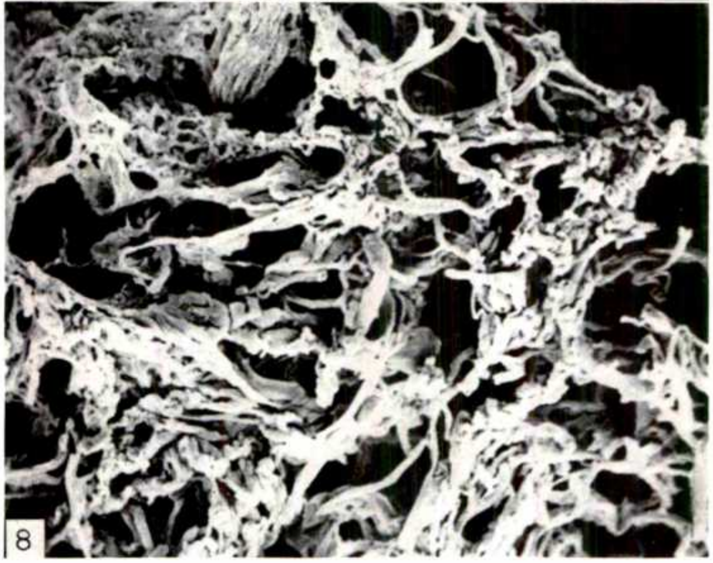


FIGURE 7. Fibres in the upper dermis converge forming a sponge-like appearance. They are running in different directions (× 400).

FIGURE 8. Higher magnification of a portion of Fig. 7. Fibres are irregularly shaped, some have pits, others are swollen and appear flimsy (× 800).

to the surface. In specimens from the neck, fibres running in different directions (more than 3 μm in diameter) in the upper dermis converged giving a sponge-like appearance (Fig. 7). The fibres were irregularly shaped; some had pits, but others were swollen and looked very flimsy (Fig. 8). Amorphous masses as seen in the hyaluroidase-treated specimens were not present. There were no differences in appearance of elastic fibres in the mid- and deep dermis between the abdomen and the neck.

DISCUSSION

The present study demonstrates the three dimensional architecture of solar elastosis and normal dermis. As compared with normal dermis, solar elastosis shows irregular and flimsy-looking fibres combined with amorphous masses. In this study, hyaluronidase digested ground substance, as Findlay (1969) reported, and autoclaving removed collagen fibres as well as ground substance.

There were differences in the appearance of solar elastosis between hyaluronidase-treated specimens and autoclaved ones. In the former, there were amorphous masses identical to elastotic material seen at the light microscope level as well as fibrous structures. In the latter, the amorphous masses were removed together with collagen fibres, leaving only fibrous structures similar to elastotic fibres seen at the light microscope level. The removal of the amorphous masses was probably due to a mechanical washing because of their fragility.

Dawber & Shuster (1971) reported SEM findings of solar elastosis, but they could not demonstrate as clear a structure as seen in the present study. This may be because they removed neither ground substances nor collagen fibres. In addition, they could not distinguish between a collagenous or an elastic origin of the elastotic deposits.

The present study indicates that solar elastosis has a nature similar to that of elastic fibres; that is, both are resistant to hyaluronidase and autoclaving. These results support the concept that the origin of solar elastosis is elastic tissue, as several other observers have suggested (Felsher, 1961; Banfield & Brindley, 1963; Smith, 1963; Mitchell, 1967).

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The potassium iodide patch test in dermatitis herpetiformis in relation to treatment with a gluten-free diet and dapsone

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SUMMARY

The potassium iodide patch test was studied in twenty-six patients with dermatitis herpetiformis. Histological assessment was found more sensitive than clinical. All of five patients with active disease and not on treatment had a positive test, whereas only two of six patients taking a gluten-free diet (GFD) and one of eight taking dapsone were positive. In another two patients taking a GFD, but in whom the diet had not been strict, the test was positive. All three patients in remission and both patients with the linear pattern of IgA (but with active disease) were negative. Immunofluorescence studies showed no difference in the presence, quantity, or distribution of immunoglobulin, complement or fibrinogen between the patch test site and uninvolved skin, or in the uninvolved skin between patients with and without active lesions.

It is well known that oral potassium iodide (KI) will aggravate dermatitis herpetiformis (DH), and KI applied to the uninvolved skin will produce clinical lesions similar to those of DH. How KI produces these effects is unknown. Both dapsone and a strict gluten-free diet (GFD) (Fry *et al.*, 1973; Reunala *et al.*, 1977) will clear the skin lesions of DH: dapsone in a few days, but a GFD in many months. How these treatments stop the skin lesions developing is also unknown. We have studied the KI test in relation to these treatments, and in patients who were untreated and those in spontaneous clinical remission, in an attempt to elucidate the factors which may be responsible for production of the skin lesions.

PATIENTS AND METHODS

Twenty-six patients were studied. The diagnosis of DH had been established by the clinical features, clearance of the rash with dapsone and relapse when the dapsone was stopped, and the presence of IgA in the uninvolved skin.

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Eight patients were being treated with a GFD alone. In six the diet was strict and there was no recent history of skin lesions. Two of the eight patients had had skin lesions within the last month and in each patient this was due to inadvertent gluten intake, but at the time of study there were no skin lesions.

Twelve patients were controlled by dapsone and one by sulphapyridine; seven were asked to stop their dapsone 1 week prior to the patch test—five were able to tolerate this, but two found the eruption intolerable and took dapsone after 5 days, so that at the time of the patch test they had taken dapsone within 48 h; these two patients have therefore been included in the dapsone group. However, in both patients skin lesions were still present at the time of the KI patch test, though new lesions were not appearing.

Three patients in spontaneous remission were studied; they no longer required dapsone or GFD and had no skin lesions. Two patients with the linear pattern of IgA were studied; they were well controlled by dapsone and stopped this drug 1 week prior to the patch test. Eight volunteer physicians in the Skin Department were also patch tested.

Potassium iodide patch test

All patients had two patches applied to the upper arm. One was 50% KI in yellow soft paraffin (YSP) and the other was YSP alone. Both patches were coded and applied by a physician who did not read the tests. The patch tests were applied for 24 h. The results were graded: no reaction, erythema, small blisters and bulla.

Biopsies

Each patient had two 4 mm punch biopsies. One biopsy was taken from the KI patch test site, and the other from clinically uninvolved skin. The biopsy from the patch test site was divided into two. Half was fixed in formol-saline and processed to paraffin. Sections were cut at four levels and stained with haematoxylin and eosin; sections at an appropriate level were stained with the Martius Scarlet Blue method and the Mallory's phosphotungstic acid haematoxylin method for fibrin (Cook, 1974). The other half was embedded in OCT (Ames) and snap frozen in liquid nitrogen, as was the biopsy from uninvolved skin. Sections were cut and examined by direct immunofluorescence for immunoglobulin and complement as described previously (Fry *et al.*, 1978). Fibrinogen was also detected by direct immunofluorescence using appropriately diluted fluorescein conjugated anti-human fibrinogen (Wellcome).

RESULTS

There were no changes at the sites of patch testing with YSP only. Five of the eight control subjects showed erythema at the site of the KI patch test, but none had blisters. Erythema was therefore considered a negative result, and blisters or bullae a positive result. The results are shown in Table 1.

One of the five controls who showed erythema on the patch test had a biopsy, which showed slight spongiosis and mild chronic perivascular inflammation; this finding was therefore considered a negative result on histology, whereas a subepidermal bulla was considered a positive result. The results are given in Table 1. In four of the ten cases positive on histology there were intra-epidermal vesicles as well as subepidermal, and in three of the ten there were collections of eosinophils in the subepidermal bullae. However, no eosinophilic micro-abscesses were found in the dermal papillae.

The immunofluorescence results are given in Table 2. IgA was found in the uninvolved skin of all patients except one, and this patient had IgA in a previous biopsy. There was no significant difference

TABLE 1. Clinical and histological results in relation to treatment

	No. of patients	No. with skin lesions at time of test	Clinical positive	Histology positive
GFD (strict)	6	0	0	2
GFD (recent gluten intake)	2	0	2	2
Dapsone	8	3*	0	1
Dapsone stopped	5	5	4	5
Remission	3	0	0	0
Linear IgA	2	2	0	0

* Two patients with active lesions took dapsone for 48 h prior to KI patch test.

TABLE 2. Immunofluorescence results in KI patch test sites and uninvolved skin

	No. of patients	Patch test site				Uninvolved skin			
		IgA	IgM	C3	Fib.	IgA	IgM	C3	Fib.
GFD (Strict)	6	5	2	3	4	5	3	0	3
GFD (recent gluten intake)	2	2	1	0	1	2	1	1	2
Dapsone	8	8	1	5	4	8	3	6	3
Dapsone stopped	5	5	0	4	4	5	2	4	3
Remission	3	3	0	1	2	3	0	1	3
Linear IgA	2	2	1	0	0	2	1	0	0

IgG was present in both the patch test site and the uninvolved skin in one patient; no other patient had IgG in either biopsy.

in the presence/absence or quantity of IgA, IgM, IgG, complement (C3), or fibrinogen between the patch site and uninvolved skin, nor was there a difference in the uninvolved skin from patients with active lesions compared with uninvolved skin from patients without active lesions. The patch site results in histology positive cases did not differ from those in histology negative cases, nor was there a difference in the patch site results from patients with active lesions compared with those from patients without active lesions.

In the uninvolved skin fibrinogen was found in fourteen patients and in all it was at the same site as the IgA. In the patch test site fibrinogen was present in fifteen patients but in only eight was it found at the same site as IgA.

DISCUSSION

These results show that histological assessment of the KI patch test produced more positive results (10) than the clinical (6) and was taken as the final result. All patients with active disease and not on treatment had a positive test (5/5), and both patients not on a strict GFD also gave a positive result, whereas only two of six patients on a strict GFD and one of eight taking dapsone or sulphapyridine had a positive test.

There was no significant difference (chi-squared test) in the frequency of a positive result in patients with active lesions (5/10), and in those without (5/16). However, four of the ten patients with active lesions who gave negative histology may have done so for specific reasons: two of the patients with active lesions were those who took dapsone 48 h before the patch test because the rash was intolerable, and a further two had linear IgA deposits which may imply a different mechanism of disease.

It is probable that dapsone in adequate dosage will suppress the KI test and the only patient with a positive histology but no skin lesions may be taking only just enough dapsone to stop clinical lesions. Conversely the patient with active lesions but no positive histology was known to have increased dapsone dosage just prior to the test.

The results in the patients taking a GFD are interesting. The four patients with positive histological results all had no skin lesions at the time of the KI test. However, two of the patients admitted to a small intake of gluten with skin lesions approximately 1 month before the test. It is possible that in all four patients with a positive histology there had been intake of small quantities of gluten. This may be enough to produce a positive KI test, but not sufficient to give skin lesions without KI challenge. Our observations suggest that the pathogenesis of a blister in DH produced by the disease process and by KI may be different as microabscesses were not found in the dermal papillae in the positive patch tests. Because of the failure to find microabscesses we cannot say that the histology of the KI produced lesion and the one produced by the disease are indistinguishable as claimed by Salo *et al.* (1970).

Mustakallio, Blomquist & Laiho (1970) reported that fibrinogen in the dermal papillae was a feature of DH lesions but was not found in uninvolved skin; despite our use of similar techniques we were unable to demonstrate a significant difference between the patch test site (fibrinogen present on immunofluorescence in 15/26) and the uninvolved skin (14/26), or between fibrinogen in positive patch sites (8/10) and in negative patch sites (7/16).

The immunofluorescence findings provided no evidence that IgG, IgM, IgA, or complement (C3) played a significant part in the production of lesions, as there was no difference in the presence, quantity or site of these between the patch tests and the uninvolved skin.

Complement was present in the uninvolved skin of fewer patients on GFD (1/8) than of patients on dapsone (6/8) or patients in the dapsone-stopped group (4/5), and this difference has been noted previously (Reunala, 1978; Haffenden, Wojnarowska & Fry, 1979). Complement does not appear to be associated with the presence or absence of active lesions as it was present in uninvolved skin in six of eleven patients with active lesions and in six of seventeen patients without active lesions (chi-squared test: no significant difference).

It would appear either that the IgA is not involved, or that other factors must also be present, in the production of the lesion by KI. IgA was present in the skin, both in the patients controlled by a GFD in whom the test was negative and in those in natural remission. The patients in natural remission also imply that factors other than gluten are important in the production of DH. These patients were taking a normal diet and yet no lesions were produced. The reason why these patients are in remission is not known, but it is possible that their skin is no longer susceptible to gluten because of a fundamental alteration in the skin itself, or there may be a more general alteration in the immunological behaviour of the patient.

How KI produces blisters in DH is not known. Other substances such as bromides and thiocyanates have also been reported to produce similar lesions (Alexander, 1975). KI is a strong oxidising agent and it may have a direct chemical effect on the connective tissue of the upper dermis, altering its structure and making it more susceptible to the factors which normally produce a DH lesion. However KI produces the lesion, its effect is blocked by dapsone and a strict GFD, and these observations may well prove useful in further studies directed at understanding the processes involved in the pathogenesis of DH.

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Pharmacology and Treatment

The local treatment of hypertrophic scars and keloids with topical retinoic acid

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SUMMARY

In a clinical trial twenty-eight intractable cases with scars were treated with daily applications of a 0·05% solution of retinoic acid.

The results were evaluated objectively and subjectively. Slight to marked reduction of the size of these scars and decrease of such complaints as itching were noted in the majority of the cases. A favourable result was obtained according to the patients in 79%, and according to the opinion of the medical examiner in 77% of the patients.

Many therapeutic modalities have been advocated for the treatment of keloids without convincing results. Multiplicity of methods of treatment is always an indication that none of them is entirely satisfactory.

It has been demonstrated that DNA dependent polymerase and RNA synthesis are accelerated after topical application of retinoic acid (Lukacs, Christophers & Braun-Falco, 1971). Since the convincing evidence presented by Christophers & Braun-Falco (1968) it has been found that retinoic acid causes a decrease of normal tonofilament and keratohyalin synthesis, coupled with an increased production of mucoid substances and epidermal cell growth rate.

Raab & Gmeiner (1976) further attempted to analyse the mechanism of action of this drug. They demonstrated an increased oxygen consumption in rat and human skin cells in the presence of retinoic acid, and therefore could state that stimulation of nucleic acid synthesis did occur in epidermal cells.

According to the autoradiographic studies of Pullman, Lennartz & Steigleder (1975) there was a definite increase in proliferation of epidermal cells, the pattern of which resembled not that of psoriasis but of a regenerative process. The *in vivo* experiments of Prutkin (1972), according to whom wound healing was enhanced by the topical application of retinoic acid, are in accordance with these laboratory findings. A comparative study was carried out by Christophers & Lagner (1974) on the *in vitro* effects of retinoic acid on cultured fibroblasts, lymphocytes and epidermal cells. They demonstrated that 'when DNA synthesis is taken as a parameter for cell growth in epidermal cells a potent stimulation is exhibited. In fibroblasts as well as in lymphocytes no stimulating effects were seen. On the contrary, the presence of retinoic acid in the culture medium caused marked *inhibition of DNA*

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synthesis'. In lymphocytes and fibroblasts it was suggested by Dr R.H.Cormane (Binnengasthuis, Amsterdam) that this inhibitory effect on DNA synthesis may be useful in the treatment of excessively growing scar tissue, although it is well known that *in vitro* effects are not always applicable to *in vivo* situations.

MATERIALS AND METHODS

Patients

The above considerations led us to start a clinical trial using retinoic acid (0·05%) as a topical application in twenty-eight patients having keloids and hypertrophic scars. (No attempt was made to differentiate between these.)

All patients were requested to apply the solution to their scars twice a day. If, however, the surrounding skin became irritated, they were asked to continue only once a day. The age of the patients ranged from 3 to 68 years, 92% of them were under the age of 36 years. Nine patients were male and nineteen female.

The cause and location of the scars, the treatment prior to retinoic acid locally, the duration and the response to retinoic acid are described in Table 1. Several patients had received Kenacort intralesionally prior to being treated with retinoic acid. All patients were seen by the author and the results of the treatment were listed as being excellent, good, fair or poor. Some patients were excluded because the period of time during which they used retinoic acid was too short (less than 3 months).

Three months after completion of this study all patients received a questionnaire requesting them to answer twelve questions about their subjective findings of the effectiveness of the drug. These questions included improvement or disappearance of pain and/or itching as the main parameters.

Preparation of retinoic acid solution

The solution of retinoic acid for topical application is commercially available (Hoffman-La Roche BV, Mijdrecht, Netherlands). The lotion contains ethanol and propylene glycol with 0·05 g retinoic acid (Tretinoin) per 100 ml.

RESULTS

Twenty-one of the twenty-eight patients cooperated in the study. The topical application of retinoic acid solution was judged (subjectively) as causing improvement by fourteen patients (79%) and no improvement by three patients (21%).

We observed very similar results objectively: the excellent, good and fair results amounting to 77% and poor and excluded patients to 23% (Table 2).

Thirteen of the twenty-one (64%) patients found that discolouration (blue, purple) was diminished and that the lesions became flatter, while two patients developed slight atrophy at the site of the scars.

Only a few of our patients complained of pain in their scars but of these three stated that their pain was cured and one (CW) that it was dramatically improved. This patient had been treated for years with all modalities of therapy for extensive scarring over most of her trunk but without any success. Now she is free of pain for the first time in years.

Some patients noticed a slight decrease of the hyperpigmentation of the scar but most noticed no change in pigmentation before or after treatment. Nearly 50% of the patients had some transient redness and/or scaliness of the skin in the treated area. The parents of one patient decided to discontinue the therapy because of these symptoms.

TABLE 1. Clinical details of patients

Patient no.	Patient initials	Sex	Age	Cause and location	Duration of treatment (months)		Response		Notes
					Kenacort prior to retinoic acid	Retinoic acid	Kenacort	Retinoic acid	
1	E.D.	F	25	Postop. breast	—	11	—	Excellent	
2	J.H.	F	30	BCG shoulder	—	7	—	Good	
3	E.H.	F	11	Postop. left leg	19	8	Fair	Good	
4	E.B.	M	36	Postop. presternal	84	4	Poor	Poor	Every kind of treatment of no avail
5	A.B.	F	15	Burn body	10	4	Fair	Good	
6	M.V.	F	17	Chickenpox body/shoulder	1 ×	2*	Good	Fair	
7	A.S.	M	8	Postop. shoulder	7	12	Fair	Good	
8	J.V.	M	20	Postinfection cheek	4	5	Fair	Fair	
9	C.W.	F	68	Postop. body	15	2*	None	Excellent	Improvement of pain
10	K.R.	F	11	Postop. body	10	3	Fair	Fair	
11	A.H.	M	17	Postop. body	10	1*	Excellent	Recent	
12	J.F.	F	11	Postop. face	14	4	Fair	Good	In combination best
13	Y.B.	F	23	Donor site graft abdomen	14	3	Good	Excellent	
14	H.J.	F	10	Postop. face	—	6	—	Good	
15	E.H.	F	34	Postop. abdomen	12	10	Fair	Excellent	Improvement of pain
16	C.H.	F	3	Burns body	—	7	—	Fair	Recent
17	A.D.	F	33	Postop. body	—	3	—	Fair	Recent
18	R.V.	M	3	Burns thorax	—	8	—	Fair	Recent
19	C.K.	F	33	Postop. abdomen	9	6	Fair	Good	Re-operation and Kenacort of no avail
20	A.V.	F	4	Chemical burns face	—	13	—	Fair	
21	E.H.	M	3	Burn body	—	12	—	Fair	Compression dressing
22	R.W.	M	14	Trauma wrist	15	9	Fair	Fair	Compression dressing
23	J.Z.	M	5	Burn leg	3	11	None	Fair	
24	M.V.	M	3	Burn body	—	8	—	Good	
25	D.S.	F	50	Donor site neck	22	22	Fair	Good	
26	E.M.	F	36	Postop. Abdomen	7	5	Fair	Fair	
27	E.R.	F	18	Trauma body	4	2*	Fair	Fair	Re-operation and Kenacort of no avail
28	H.D.	F	22	Postop face	8	5	Fair	Fair	

* These patients are excluded from Table 2 as the duration of treatment was less than 3 months.

TABLE 2. Evaluation of the results of the treatment with retinoic acid (0·05%) solution, topically applied on scars

Excellent	Good	Fair	Poor	Excluded
4	11	8	1	4
Total number of patients 23 (77%)			Total number of patients 5 (23%)	

In eleven cases the frequency of application was changed from twice daily to once daily and in one patient it was changed to once every 2 days whereafter no discomfort was noted.

DISCUSSION

Some authors state that the difference between keloids and hypertrophic scars can be defined by the fact that keloids grow beyond the boundaries of a scar and the other variety does not (Ketchum, Cohen & Masters, 1974). However, Cohen *et al.* (1974) found that in spite of a proven increased collagen synthesis in both types of scars, the collagen synthesis at the edge of a keloid was normal.

It has been established that proline hydroxylase activity is higher in hypertrophic scars and still higher in keloids as compared to so-called normal scars (Cohen, Keiser & Sjoerdsma, 1971).

With conventional histological examination it is still very difficult, if not impossible, to differentiate keloids from hypertrophic scars (Conway *et al.*, 1972). Therefore we considered them as one group.

Through the centuries many substances have been applied to keloids to improve their appearance and/or to alleviate the symptoms they may cause. To name some of these substances: vitamins (E), enzymes, fibrolysin, hyaluronidase (Cosman *et al.*, 1961), tetrahydroxyquinone, N-acetylhydroxyproline (Koonin & Charles, 1975), Benadryl (Cohen *et al.*, 1972), iodine, formalin, nitrogen mustard etc. The results were variable or nil.

During the past 15 years the effectiveness of triamcinolone injections locally, whether or not combined with surgery, has been established by Griffith (1966), Vallis & Lynn (1967), Ketchum, Robinson & Masters (1971) and Rook (1972).

Radiation, mostly in the form of X-rays or radium, is also a well known remedy. Since radiation does not affect mature fibroblasts, it is useful only if applied within 24 h following surgery. Considering the long-term effects of radiation therapy some of our colleagues vigorously object to any kind of radiation therapy to a relatively benign lesion.

Local pressure to prevent or cure elevated scars has also proved its value. The mechanisms were discussed by Linnares *et al.* (1973).

A disadvantage of most of the methods described so far is their inconvenience.

This article is the first preliminary report of our clinical trial using a topical application of retinoic acid for the treatment (and palliation) of hypertrophic scars and keloids. A further aspect of this therapy is the possible effect of retinoic acid on malignant cell growth. Shamberger (1971) reported a marked reduction of the incidence of induced skin tumours by the local application of this drug.

Prutkin (1975) reported his results in experimentally induced epithelial tumours of rabbits' ears treated with retinoic acid 0·1% and/or 5-fluoro-uracil 2%. He concluded that the therapeutic effect is strongly dose-dependent. Therefore we intend in our future studies to change the concentration of retinoic acid gradually up to 1%.

Intralesional injection will be considered also (Dermo-jet, Robbins Instruments Co.). Sterilization of the drug can be achieved by using a millipore filter, since heating will result in disintegration of the active ingredients. Injection into healthy tissue should not be advocated.

In this series there were three intractable cases of keloids who had previously been treated with all possible modalities of therapy (release of tension, Z-plasty, skin grafting, X-ray, pressure, intralesional steroids, etc.) without any improvement. In two of these patients, who were of partly oriental origin, retinoic acid had no effect, in the third patient who was of caucasian origin it showed marked improvement.

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Case Reports

Cutaneous carcinoma arising in erosive pustular dermatosis of the scalp

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SUMMARY

We report a patient in whom a histologically unusual carcinoma has arisen in an area of erosive pustular dermatosis of the scalp.

CASE REPORT

The patient, a 77-year-old housewife, had, at the age of 20, caught a wisp of hair in a machine spindle which tore off virtually all her scalp. Attempts at replacing the scalp were unsuccessful, and she received numerous skin grafts to the denuded area over a period of 9 months. For the subsequent 50 years, her husband cleaned the area daily with dilute mercuric nitrate in yellow soft paraffin and applied paraffin gauze. In spite of this treatment, the scalp never healed totally. During this period small areas of painful pustulation developed on the scalp. These, however, healed spontaneously.

During the 18 months prior to presentation in 1979, an extensive and painful area of pustulation developed on the scalp, with a reddened nodule over the right forehead. The pustulation failed to respond to topical antibiotics (Soframycin and sodium fusidate).

Examination revealed almost total hair loss on the scalp with destruction of the follicles, extensive and tender superficial pustulation with easily removed crusts of macerated keratin, and areas of indentation of the scalp. Over the right frontal region of the scalp there was a reddened nodule with a rolled edge (Fig. 1).

A full blood count was normal; W.R. and V.D.R.L. and auto-immune profile were negative. Repeated cultures of the pustular areas grew only *Staphylococcus albus* and diphtheroids. A skull X-ray showed increased bone over the left postero-occipital region, presumably incorporated periosteal bone following chronic infection. There was also a dural plaque in the left frontal region, but no evidence of bone involvement from the carcinoma. Patch testing to the European standard battery and to soframycin, sodium fusidate and mercury yielded only a (1 +) positive reaction to formalin.

A skin biopsy taken across the edge of the nodule showed crusting and inflammation of the horny layer overlying a patchy, chronic, inflammatory infiltrate in the dermis comprising lymphocytes and macrophages with numerous plasma cells. Arising from this area was an ulcerated tumour (Fig. 2),

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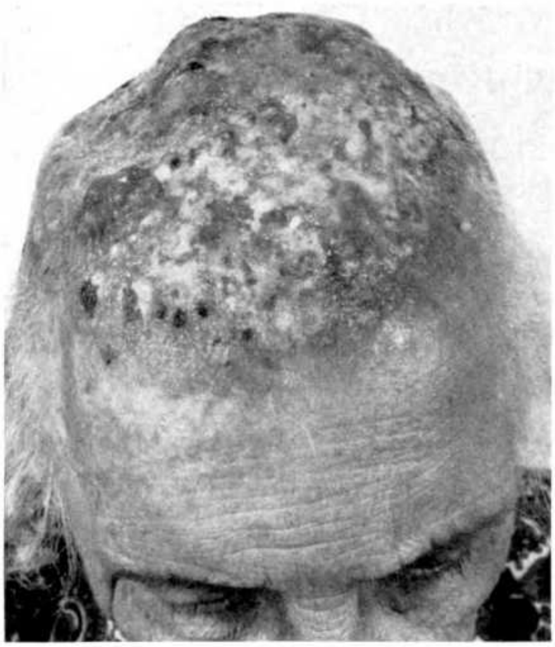

FIGURE 1. Scalp showing extensive pustulation and indentation. An ulcerated tumour is arising in the right frontal region.

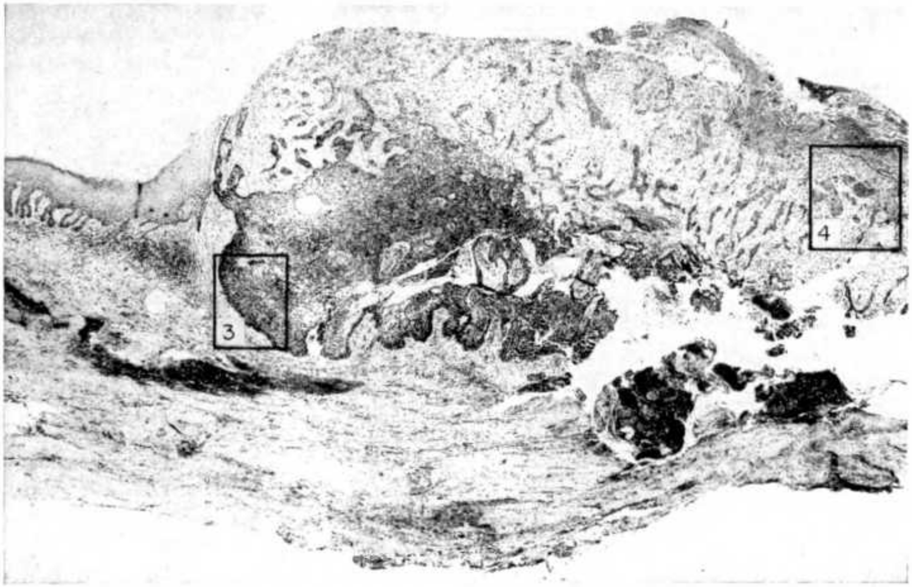

FIGURE 2. Section of scalp lesion showing dermal inflammatory infiltrate adjacent to ulcerated tumour (H & E, × 24).

whose deeper part showed basal cell differentiation with palisading (Fig. 3). The more superficial part showed complete squamous differentiation with strands of neoplastic squamous epithelium separated by oedematous stroma (Fig. 4).

The scalp became less painful after careful removal of the crusts and gentle irrigation with hydrogen peroxide, and the pustular condition has improved with the regular use of 0·05% clobetasol propionate (Dermovate) scalp application. A course of radiotherapy with electrons (6000 rad in twenty treatments over 28 days from a Betatron) led to resolution of the tumour with no sign of recurrence after 6 months.

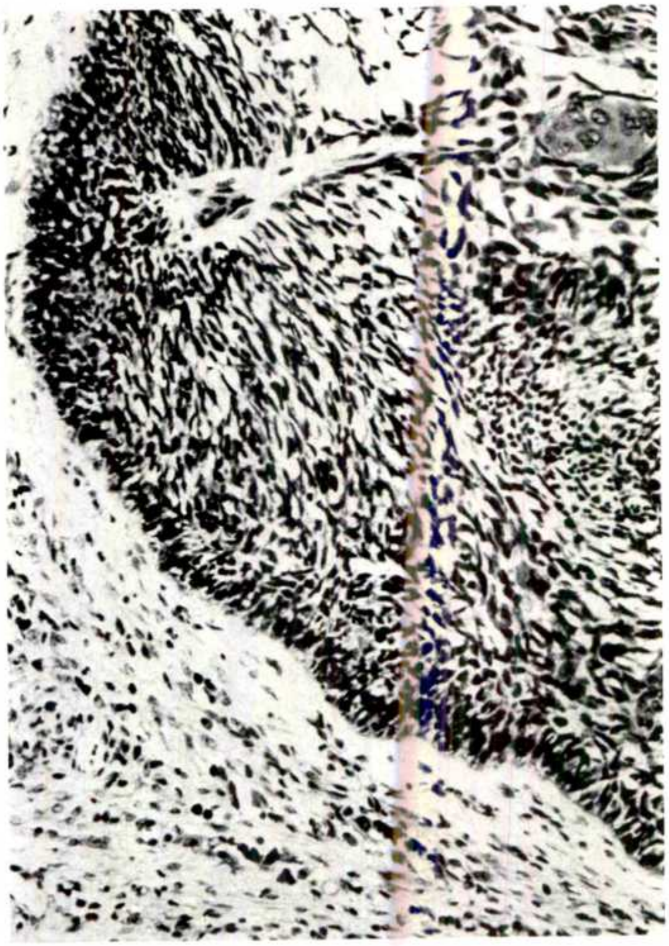

FIGURE 3. Section of Fig. 2. showing tumour differentiated as basal cell carcinoma (H & E, × 180).

DISCUSSION

The clinical and histological appearances of the inflammatory scalp condition are closely similar to those reported as erosive pustular dermatosis of the scalp, in six elderly females by Pye, Peachey & Burton (1979). Those patients also presented with localized areas of superficial crusting and flaccid pustulation, leading to scarring and alopecia. The investigations, as in this case, were essentially negative and histological changes were non-specific. Unlike most of their patients, in whom the

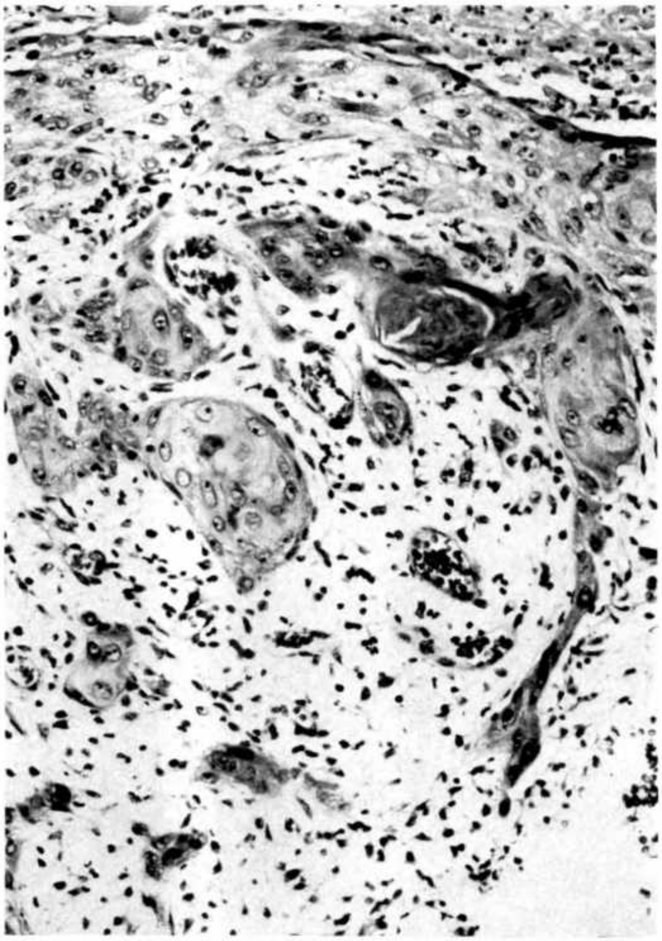

FIGURE 4. Section of Fig. 2 showing tumour differentiated as squamous cell carcinoma (H & E, × 180).

aetiology of the condition was unknown, our patient gives a definite history of extensive trauma 50 years previously. Similarly, a history of previous minor scalp lacerations was also noted in one of the patients described by Pye *et al.* (1979).

Carcinoma formation has not previously been described in patients with this disorder although it is a recognized complication of chronic ulcers and old burn scars. The tumour which occurred in our patient is unusual histologically, showing typical features of both basal cell carcinoma and squamous carcinoma. As this raises the possibility that erosive pustular dermatosis of the scalp is pre-malignant, patients with this condition should be kept under review for a long period.

ACKNOWLEDGMENT

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Systemic mastocytosis with diffuse crocodile-like pachydermic skin, pedunculated pseudofibromas and comedones

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SUMMARY

A case of systemic mastocytosis is described, characterized by the severity of its cutaneous manifestations (diffuse encephaloid pachydermia, numerous skin tumours reminiscent of molluscum fibrosum, and large comedones) associated with gastro-intestinal disturbances and high blood histamine values but running a benign clinical course.

In cutaneous mastocytosis systemic involvement of organs other than skin is rare. However, since the description of a case by Touraine, Solente & Renault (1933) and reports of other cases by Bertellotti (1943), Ellis (1949), Degos *et al.* (1952), Sagher *et al.* (1956), Meneghini & Hofmann (1961), Mutter, Tanneubaum & Ultmann (1963), Sagher & Even-Paz (1967) and by other authors, the condition has been well recognized. In most cases systemic mastocytosis is limited to the involvement of skin and bones. In the rare cases of mast cell infiltration of other organs, especially liver, spleen and bone marrow, the disease is potentially serious and even fatal within several years.

The unusual clinical aspects of a case of systemic mastocytosis under our care and its unusual course prompted us to present this brief report.

CASE REPORT

A 52-year-old woman has been attending our out-patient clinic since 1976 with a dermatosis dating back to the age of approximately 2 months. The patient remembered being told by her parents that already at that age her skin was brownish, showed small diffuse raised lesions and that she had attacks of itching. From a very young age she also suffered frequent episodes of diarrhoea. In the course of years the skin changes spread to involve the trunk and limbs but there was no gross systemic disturbance. In May 1959 the patient was admitted to the Institute of Medical Pathology of our University for investigation of the clinical picture which had developed over the 3 preceding years and consisted of debility, moderate pyrexia and a sensation of heaviness in the left hypochondrium. At this stage

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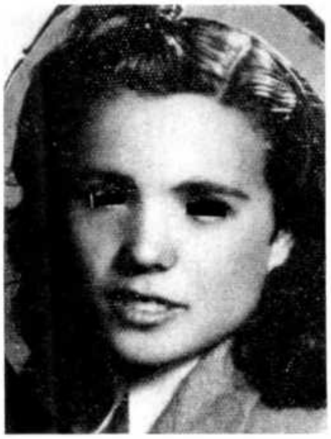

FIGURE 1. The patient at age 19.

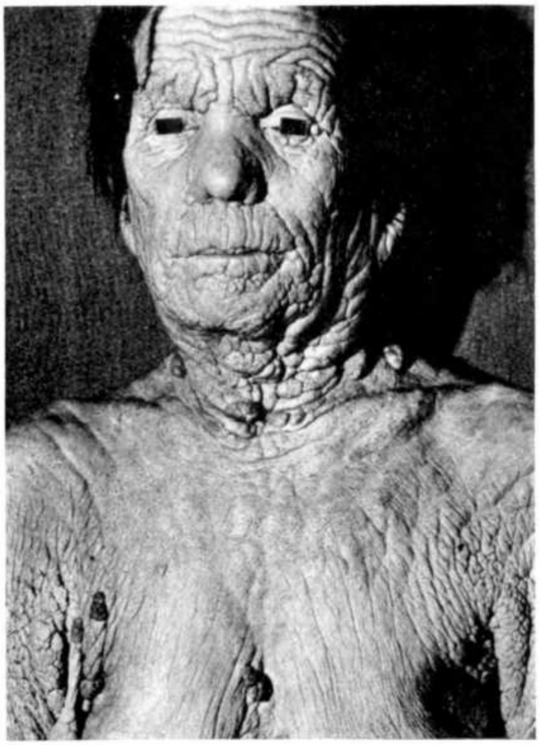

FIGURE 2. The patient at age 52. Pachydermia, pedunculated pseudofibromas and large comedones.

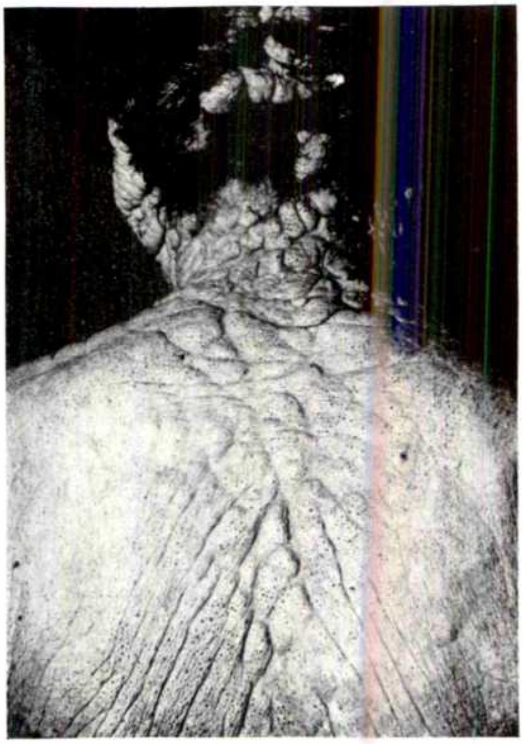

FIGURE 3. Appearance of the lesions on the back and hairy skin areas.

the signs of hypersplenism with haemolytic anaemia and leucopenia led to splenectomy. Following histological examination of the spleen and liver fragments removed during the operation and of a skin biopsy, systemic mastocytosis was diagnosed. Numerous mast cells were also demonstrated in bone marrow smears obtained by sternal puncture. The patient was discharged 25 days after the operation in good general condition and returned to normal life. The case was reported by our University pathologists (Chieco-Bianchi & Albano, 1963) as one of systemic mastocytosis; at that stage the skin was brownish, very thick deeply wrinkled or embossed, mainly round the neck, in the axillae and in the inguino-femoral areas.

In the subsequent few years the dermatosis, subjectively troublesome because of episodes of pruritus, showed further deterioration until the present condition has been reached, with the skin still more thickened and deeply embossed with frankly a pachydermic appearance (Figs 1–3). The condition spread to the face and hairy skin areas. Numerous molluscum-like tumours appeared symmetrically over the neck (including the nape of the neck), back, face, chest, axillae, forearms and pubic and inguino-femoral regions. The lesions were either lobulated, nodular or flat, varied in size and were covered with yellowish or brownish skin, either hard or soft. The general appearance of these lumps varied from that of encephaloid tumours to structures reminiscent of bunches of small grapes. The deeply embossed skin showed also other isolated, either sessile or pedunculated molluscum fibrosum-like lesions and blackheads or large comedones in the hair follicles. Briefly, the appearance was that of crocodile skin. Dermographism was very evident. Duodenal ulceration has been present for 3 years.

Of the many test results obtained particularly interesting seemed the hypergammaglobulinaemia (IgG 2750 mg/100 ml; IgA 720 mg/100 ml; IgM 290 mg/100 ml) and the repeatedly raised blood

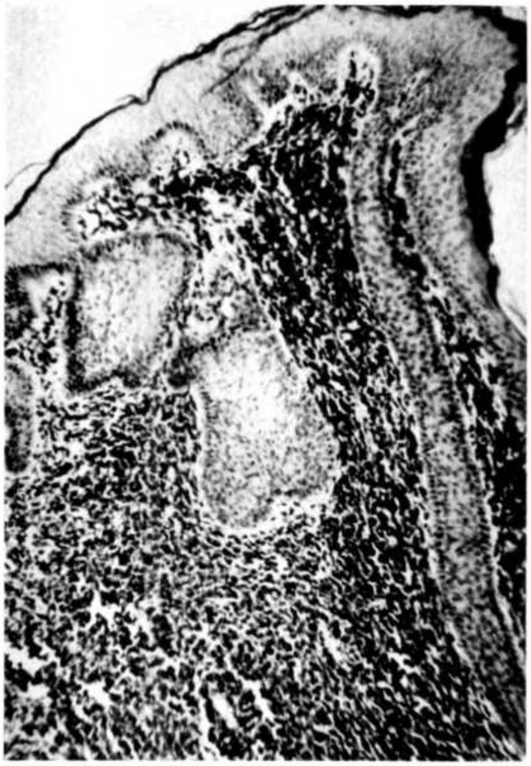

FIGURE 4. Histopathology of a molluscum-like tumour. Massive infiltration of mast cells in superficial and deep dermis (Toluidine blue, × 90).

histamine level: 0·093 μg/ml (spectrofluorometric method of Shore *et al.* (1959): normal values 0·04–0·07 μg/ml). Radiological studies demonstrated a duodenal ulcer and an ivory osteoma of the frontal bone. The karyotype was normal. The radiological appearances of the chest and the results of the skeletal survey were normal as were the blood count, ophthalmological examination, blood levels of T_3 and T_4, 17-ketosteroids and 17-hydroxycorticosteroids, kidney and liver function tests and neurological examination. The histology (Figs 4–6) with toluidine blue, Giemsa and Gomori stains is characterized by massive accumulation of mast cells of various types, elongated spindle or cuboidal in shape. Basophilic and metachromatic granules are chiefly present in the cytoplasm. The infiltrate extends through the dermis and subcutis.

COMMENT

The case history of our patient combined with the clinical, laboratory and histological findings confirmed beyond all doubt the diagnosis of cutaneous and visceral mastocytosis. The diffuse visceral involvement was evidenced by the enlargement of the liver, the mastocytic infiltration of the bone marrow and the gastro-intestinal disturbance. In contrast, the skeletal changes reported in association with systemic mastocytosis were conspicuous by their absence.

The blood levels of histamine, checked repeatedly, were invariably and significantly raised, as previously reported in some cases of cutaneous mastocytosis including its diffuse forms (Meneghini & Hofmann, 1961). This high histamine level might have been responsible not only for the pruritic

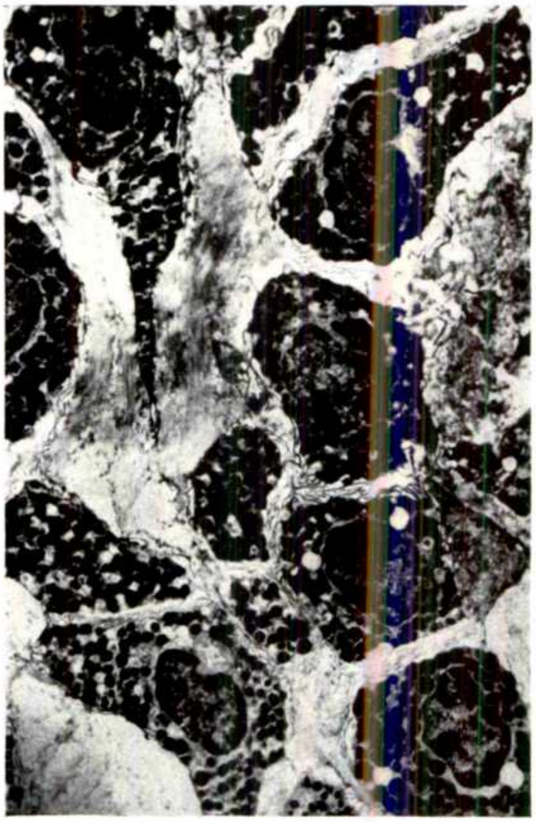

FIGURE 5. Electron micrograph showing mast cells (× 2400).

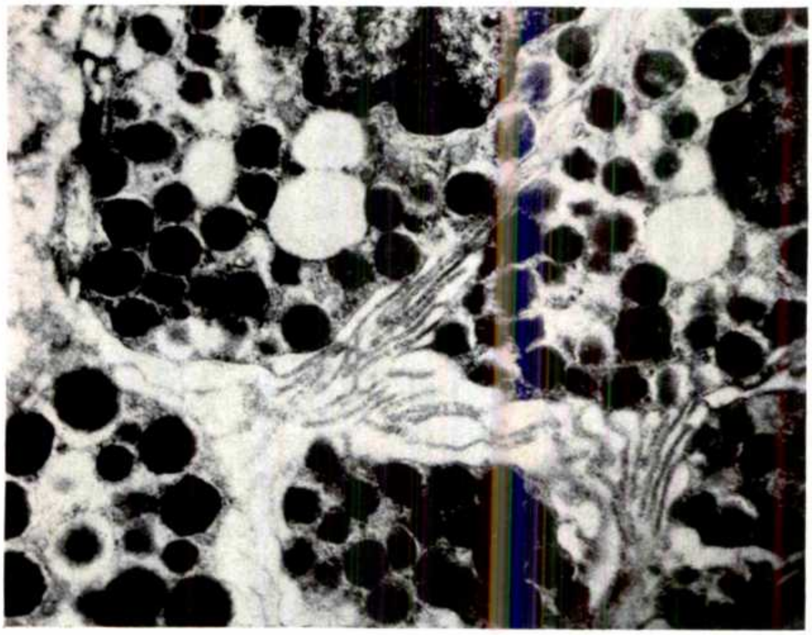

FIGURE 6. Electron micrograph showing higher magnification of granules in mast cells (× 17,000).

urticarial reactions, the congestive vasomotor crises and the periodic headaches complained of by the patients, but also for the gastro-intestinal disturbances and the duodenal ulceration (Rebhun, 1979); histamine is a well known powerful stimulant of gastric secretion.

What seemed unusual in our patient was the evolution of the skin appearance towards the deeply embossed crocodile-like pachydermia and the development of numerous comedones and pedunculated pseudofibromas. Very surprising also was the slow and relatively benign clinical evolution of this mastocytosis, with the general condition of the patient remaining unchanged and her normal working life being left, at least up till the time of writing, practically unaffected.

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British Journal of Dermatology (1980) **103**, 335.

Review Article

The cutaneous manifestations of paraproteinaemia. I

Paraproteins that result from a monoclonal proliferation of immunoglobulin-secreting cells (B cells) are associated with a wide variety of skin disorders. In certain instances, the pathogenetic role of the paraprotein is well established. In other cases the associations remain unexplained. This review attempts to classify these disorders with particular regard to the mechanisms that are thought to underlie the clinical conditions (see Appendix). The terms 'M' component disease, paraproteinaemia and monoclonal gammopathy are used synonymously to indicate excess immunoglobulin production by a clone of B cells without specifying whether this activity is benign or malignant.

The presence within the skin of paraprotein-producing cells (specific B-cell infiltrates) provides an association which is readily comprehensible but rather uncommon. Cutaneous extramedullary plasmacytomas seldom elaborate 'M' components (Canlas, Dillon & Loughrin, 1979), and more generalized lymphoproliferative disorders involving the B-cell series are characterized by the absence of cutaneous involvement. Skin deposits in multiple myeoloma occur only as a terminal event and cutaneous plasma cell infiltrates in Waldenström's macroglobulinaemia are exceptionally rare. By contrast, lymphoproliferative disorders involving T cells commonly involve the skin. Indeed, the presence of erythroderma in chronic lymphatic leukaemia indicates that the leukaemia is of T-cell rather than the usual B-cell origin (Lukes *et al.*, 1978). Clearly, T cells are not normally associated with paraproteinaemia, but occasionally monoclonal gammopathies do occur with non B-cell lymphoproliferative disorders, having been recorded twice in Sézary's syndrome (Kovary *et al.*, 1977; Joyner *et al.*, 1979). Although this association is not readily explicable, Sézary cells do exhibit helper activity (Broder *et al.*, 1979), so M components may arise as a result of T/B cell interactions (Waldmann, 1979).

A second well-defined process is the production of amyloid in plasma cell dyscrasias. Molecular weight determination, peptide mapping, and amino-acid sequence analysis have demonstrated that the light chains found in the serum and urine are structurally identical with the amyloid fibrils in the cutaneous and systemic deposits of myeloma-associated amyloidosis (Glenner *et al.*, 1971; Terry *et al.*, 1973). By contrast, the amyloid fibrils in patients with systemic amyloidosis secondary to other disease processes, such as chronic infection or rheumatoid arthritis, are unrelated to any known immunoglobulin or light chain fragment. The pattern of organ involvement in cases of systemic amyloidosis is not a reliable guide to the type of amyloid protein being produced, and mixed patterns are not uncommon (Isobe & Osserman, 1974). Nevertheless, mucocutaneous involvement would be most unusual in secondary amyloidosis, whereas the combination of macroglossia and skin deposits is highly characteristic of plasma cell dyscrasia-associated amyloidosis (Barth *et al.*, 1969). The clinical features have been well described elsewhere and do not require further elaboration (Breathnach & Black, 1979). Since circulating light chains and Bence Jones proteinuria indicate a poor degree of plasma cell differentiation, amyloid deposition carries a correspondingly poor prognosis. Patients with myeloma-associated amyloid have a median survival time from diagnosis of only 4 months (Kyle & Bayrd, 1975). Even in patients with primary systemic amyloidosis who exhibit anomalous serum

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immunoglobulins, but no other evidence of overt myelomatosis, the median survival time is only 14·7 months. Occasional failure to detect anomalous proteins in the serum of such patients may depend upon the sensitivity of the method used (Glenner, Terry & Isersky, 1973). Nevertheless, cases have been recorded without detectable immunoglobulin fragments, no evidence of a plasma cell dyscrasia, other than amyloid deposition, and a long survival time (Crow, 1977). The term 'primary systemic amyloidosis' should perhaps be reserved for such cases.

A third readily comprehensible association occurs when paraproteins are present in large amounts and produce features of hyperviscosity. Similarly, increased susceptibility to infection depends in part on reciprocal depression of other immunoglobulin classes. Both these conditions are more often associated with malignant plasma cell dyscrasias, since they occur with high rates of paraprotein production or a large B-cell mass.

Clinical syndromes which depend upon the immunological properties of a paraprotein comprise the fourth group of disorders. Paraproteins which display antibody activity, form immune complexes, and activate complement, are particularly important. Many of the syndromes, therefore, arise indirectly and are not specific markers for paraprotein production. Furthermore, the clinical lesions seldom indicate whether the gammopathy is benign or malignant, since a paraprotein may display biological activity irrespective of how it has been produced. Examples include antilipoprotein and antiglobulin activity, binding of cell surface antigen as in cold agglutinin disease, and the ability of paraproteins with anticlotting factor activity to interfere with normal haemostasis (Seligmann & Brouet, 1973).

The fifth group of disorders includes those conditions in which a pathogenetic role for the paraprotein is not established. The distinction between the last two groups is of necessity somewhat arbitrary and may need to be modified in the light of new information. Our present understanding of such mechanisms in relation to vasculitis is the subject of this article.

PARAPROTEINAEMIA AND VASCULITIS

Complement activation

Paraproteins produce vessel damage by a variety of mechanisms, not all of them immunological. Blood flow is sluggish in the hyperviscosity syndrome, vessel blockage may occur with cryoproteins that precipitate at physiological temperature, purpura can result from friable vessels weakened by amyloid deposition, and vessel changes analogous to the dermal microangiopathy found in diabetic patients have also been reported (Valdes *et al.*, 1976). However, vascular injury is usually the result of complement mediated inflammatory processes within or around the vessel wall. The ability of paraprotein to activate complement depends upon the class and sub-class of the immunoglobulin, and its tendency to form aggregates, immune complexes, or cryoprecipitates. Classical pathway activation depends upon the ability of immunoglobulins to fix the first component of complement, and this is strongest for IgG_3 and IgG_1, weaker for IgG_2, and does not occur with IgG_4 (Augener *et al.*, 1971). The ability of IgM to bind C1 increases with higher weight polymers, so that 19S IgM exhibits fifteen times the binding capacity of monomeric 7S IgM molecules, whilst IgM aggregates with a sedimentation coefficient greater than 35 exhibit seven times the binding capacity of 19S IgM (Augener *et al.*, 1971). IgA does not activate the classical pathway, but alternative pathway activation occurs with IgA_1, IgA_2 and IgG_4 (Spiegelberg, 1974). Complement activation can be enhanced *in vitro* by heat aggregation or cryoprecipitation, so the tendency of monoclonal proteins to form aggregates or cryoprecipitates is related to complement consumption *in vivo* (Naish, Collins & Barratt, 1977). However, immune complexes are also potent activators of complement via either the classical or the alternative pathways, so paraproteins with antibody activity are particularly associated with complement-mediated inflammatory processes. Antiglobulin activity is especially important in this context, since complexes that result from combination of IgM or IgA with native IgG tend to form cryoprecipitates, which again enhances complement activation (Wilson *et al.*, 1977). IgG paraproteins may also display rheumatoid factor activity, but IgG–IgG complexes tend not to be cryoprecipitable, and are most easily detected as intermediate size (13S) complexes by analytical ultracentrifugation (Kyle *et al.*, 1971).

CRYOGLOBULINAEMIA

General considerations

Cryoglobulins are either single component or mixed, and mixed cryoglobulins have a monoclonal or a polyclonal antiglobulin component (Grey & Kohler, 1973). These have been designated Types I, II and III respectively (Brouet *et al.*, 1974). Lymphoproliferative disorders can give rise to Type I or Type II cryoglobulins, but not Type III. Between 5% and 10% of myeloma proteins and macroglobulins are cryoprecipitable (Grey & Kohler, 1973). In addition, a proportion of patients with Type II cryoglobulinaemia are found to have Waldenström's macroglobulinaemia (Bonomo *et al.*, 1970) or an IgA producing B-cell dyscrasia (Aizawa *et al.*, 1979). Protein electrophoresis is not a reliable method of detecting the M-component in such cases, since interaction with polyclonal IgG can mask the monoclonal nature of the paraprotein. Demonstration of antiglobulin monoclonality is best achieved using light chain typing, but not all monoclonal antiglobulins are the result of lymphoproliferative disorders. Type II and Type III cryoglobulins may occur as a primary event or in association with a variety of auto-immune and infective disorders. Viral diseases such as infectious mononucleosis or hepatitis-B seem to be particularly important in the pathogenesis of mixed cryoglobulinaemia (Wands, Perrotto & Isselbacher, 1976; Levo *et al.*, 1977).

Type II cryoglobulins are, therefore, associated with lymphoproliferative disorders as well as with viral infection and this provides some insight into the relationship between immune complex diseases, lymphomatous transformation, and the putative role of viral agents (Miller, 1967; Anderson & Talal 1971; Levy, 1974). Animal models, such as NZB mice (Mellors, 1966) and Aleutian mink disease (Porter, Dixon & Larsen, 1965), have been extensively investigated, but the exact immunological mechanisms that operate in humans are seldom studied sequentially. The case reported by Aizawa *et al.* (1979) is therefore of interest. A patient with a history of vasculitis associated with IgA–IgG cryoglobulin, who presented with generalized lymphadenopathy and other features of Sjögren's syndrome, ultimately developed an immunoblastic sarcoma of immunoglobulin-producing cells which elaborated an IgA 'M' component with antiglobulin activity. This can be interpreted in two ways. If malignant transformation can result from chronic immunostimulation, then the lymphoma can be regarded as secondary to the connective tissue disorder. A more appealing explanation is that a B-cell dyscrasia is present throughout, and the production of a paraprotein with antiglobulin activity accounts for the associated features of mixed cryoglobulinaemia and vasculitis. Of fundamental importance, however, is the stimulus that induces monoclonal antiglobulin production initially. One hypothesis, for which there is some experimental support, is that native IgG is altered by combination with an infective agent and this induces antiglobulin production (Herd, 1973a,b). In these circumstances, however, one would normally expect antiglobulin production to be polyclonal. An alternative explanation has come with the realization that certain rheumatoid factors carry specificity for antigens other than gammaglobulin, particularly nuclear antigen (Hannestad & Stollar, 1978) and that antigenic nuclear determinants may result from invasion of lymphocytes by infective agents such as EB virus (Alspaugh *et al.*, 1978; Williams, 1979). On this basis the primary specificity of rheumatoid factor would be nuclear antigen, the antiglobulin activity could be regarded as a cross reaction, and a unifying concept for the association of immune complex disease and lymphomatous transformation is provided by the presence of an infective agent.

Clinical features

Patients with cryoglobulinaemia form a distinct group clinically as cold sensitivity may be a prominent feature. However, it is also a variable feature and depends upon the type of cryoglobulin produced and the temperature at which cryoprecipitation occurs. Single component cryoglobulins that are present in large amounts and precipitate at physiological temperature can be expected to produce clinical lesions simply by vessel blockage. Features that could reasonably be ascribed to this mechanism include Raynaud's phenomenon, digital infarcts, peripheral gangrene and necrotic lesions on exposed sites such as the nose or pinnae (Ellis, 1964). Since this phenomenon depends upon the physical, rather than the immunological, properties of the paraproteins, a similar clinical picture can be produced by unrelated cryoproteins such as monoclonal cold agglutinins, which may also produce stasis.

Cold sensitivity is a more variable feature with Type II cryoglobulins and seldom occurs with Type III cryoglobulins. *In vitro* experiments have demonstrated that the antiglobulin activity of the monoclonal component is increased at lower temperature (Capra, Winchester & Kunkel, 1969), so logically cold exposure will tend to increase immune complex formation *in vivo*. However, clinical activity may be influenced by a variety of other factors. Exacerbation of cryoglobulin-induced vasculitis occurs during intercurrent infection (Brouet *et al.*, 1974) so the ability to clear such complexes may be of equal if not greater importance.

Clinical features associated with mixed cryoglobulinaemia are presumed to result from a complement mediated vasculitis and include purpura, arthralgia, and glomerulonephritis. Even Raynaud's phenomenon is probably mediated immunologically, since it also occurs in many other forms of immune complex disease where cryoglobulins are not in evidence, and can be related to the level of phagocytosed immune complexes in circulating polymorphs (Van de Meulen *et al.*, 1979). Glomerulonephritis occurs in some 25% of cases and will affect the prognosis adversely. By contrast, renal involvement is not a feature of Waldenström's benign hypergammaglobulinaemic purpura where the antiglobulin is IgG, the complexes do not form cryoprecipitates, and the prognosis is more favourable (Kyle *et al.*, 1971). Indeed, cryoglobulinaemia has been claimed as a more accurate prognostic indicator in renal disease than the serum complement levels (McIntosh *et al.*, 1975), and the presence of a mixed cryoglobulin in Waldenström's macroglobulinaemia will affect the prognosis accordingly. One other feature that helps to distinguish cryoglobulinaemic vasculitis is the presence of a peripheral neuropathy resulting from involvement of the vasa nervorum (Cream, 1976). Hypocomplementaemia is not invariable, but methods of assessing the rate of complement consumption rather than mere complement deficiency would be of value if they were routinely available.

Single component cryoglobulins may also activate complement, so purpuric vasculitis and even glomerulonephritis have been recorded with monoclonal IgG paraproteins which cryoprecipitate but show no evidence of antiglobulin activity (Bengtsson *et al.*, 1975). This observation doubtless accounts for the clinical overlap between the different classes of cryoglobulin (Brouet *et al.*, 1974).

Several other clinically distinct conditions with the histological features of leucocytoclastic vasculitis have been described in patients with paraproteinaemia and/or cryoglobulinaemia, including urticaria, angio-oedema and erythema elevatum diutinum. These will now be considered separately.

URTICARIA AND ANGIO-OEDEMA

It is not uncommon for patients with circulating immune complexes to develop lesions of urticaria or angio-oedema. In systemic lupus erythematosus, serum sickness or Henoch-Schönlein purpura, other cutaneous lesions usually co-exist, so one could regard the urticaria in these cases as a manifestation of mild vessel damage with purpura and epidermal necrosis at the other end of the spectrum.

Occasionally patients presenting with urticaria alone are found on biopsy to have a leucocytoclastic vasculitis (Soter, 1977), and again, circulating immune complexes would account for the hypocomplementaemia seen in these cases. The single report of urticarial vasculitis in association with an IgA myeloma (Highet, 1980) could best be regarded as another example of the association between immune complex disease and malignant lymphoproliferative disorders.

Patients with cryoglobulinaemia may also present with urticaria, and since cold sensitivity may be a prominent feature, some 20% of cases of cold urticaria are associated with the presence of circulating cryoglobulins (Houser *et al.*, 1970). Again histological studies show that vasculitis and purpura may coexist (Koda *et al.*, 1978). The association of cold urticaria with single component cryoglobulins has been particularly well studied and passive transfer of cold urticaria has been successfully achieved using purified cryoprecipitates (Rawnsley & Shelley, 1968; Costanzi, Coltman & Donaldson, 1969; Koda *et al.*, 1978).

The immunological processes that underlie urticaria and angio-oedema are essentially similar. It is therefore of considerable interest that some cases of cold urticaria and acquired angio-oedema are not only associated with anomalous immunoglobulin production, but exhibit a distinctive complement profile, characterized by deficiency of C1 esterase inhibitor. The association of familial angio-oedema with an inherited deficiency of C1 esterase inhibitor is well known (Donaldson & Evans, 1963). Cases of acquired C1 esterase inhibitor deficiency are distinguished by high serum C1 fixing activity *in vitro*, depletion of the early components of the classical complement pathway *in vivo* and normal levels of C3 and C5 to C9. Although it is tempting to postulate that the urticaria or angio-oedema is secondary to C1 esterase inhibitor deficiency, it is more likely that both result from classical pathway activation and secondary consumption of C1 esterase inhibitor. This phenomenon is not unique to a particular dysglobulinaemia, having been recorded with low molecular weight (7S) IgM (Caldwell *et al.*, 1972; Hauptmann *et al.*, 1976), single component IgG cryoglobulins (Costanzi *et al.*, 1969; Hauptmann *et al.*, 1975), an IgA monoclonal gammopathy (Cheesbrough & Kinmont, 1978) and monoclonal mixed cryoglobulins (Casali *et al.*, 1978) of which two were essential and three associated with Waldenström's macroglobulinaemia. In one case of angio-oedema, immunoglobulin was demonstrable on the surface of abnormal lymphoid cells capable of activating complement and producing C1 esterase inhibitor deficiency (Schreiber *et al.*, 1976).

Patients with a variety of other clinical conditions including systemic lupus erythematosus (Ruddy & Austen, 1972), cold agglutinin disease (Wallington, French & Reeves, 1978) and diffuse plane xanthomatosis with paraproteinaemia (Jordon *et al.*, 1974; Russell Jones *et al.*, 1979) may also exhibit C1 esterase inhibitor deficiency, so the association with angio-oedema may simply reflect an increased tendency to measure this particular complement component in such cases. The association between generalized angio-oedema and paraproteinaemia is not understood and is, therefore, considered separately under the title 'systemic capillary leak syndrome' (Atkinson *et al.*, 1977).

ERYTHEMA ELEVATUM DIUTINUM

Erythema elevatum diutinum is a clinically distinct eruption with the histological features of a leucocytoclastic vasculitis. Although the lesions may be exacerbated by cold, cryoglobulinaemia has been recorded on only one occasion (Morrison, Hull & Fourie, 1977). This patient developed fixed urticarial lesions in response to cooling, accompanied by a fall in the serum total haemolytic complement. In fact, sera from most cases of erythema elevatum diutinum exhibit high C1q binding activity (Katz *et al.*, 1977). Most of the paraproteins reported in association with erythema elevatum diutinum have belonged to the IgA class (Duperrat & Rappaport, 1959; Sams, Harville & Winkelmann, 1968; Wolff, 1971; Stevanovic, 1971; Kovary, Dhonau & Happle, 1977; Archimandritis *et al.*, 1977; Katz *et al.*, 1977). In view of the persistent nature of the condition, it is perhaps not surprising that such cases do not show complement deficiency. However, *in vitro* studies would be helpful in establishing whether these paraproteins are capable of complement activation. Some patients with erythema elevatum diutinum have a history of recurrent bacterial infection and display hypersensitivity to intradermal injection of streptococcal antigen (Cream, Levene & Calnan, 1971; Katz *et al.*, 1977), so it would also be interesting to learn if paraproteins from patients with erythema elevatum diutinum exhibit antistreptococcal activity.

MISCELLANEOUS

Sporadic reports have appeared in the literature of M-component disease occurring in association with cutaneous eruptions that could be attributed to a vasculitic process, such as erythema annulare (Summerly, 1964) and erythema nodosum (Meiers, Orfanos & Schilling, 1967). Although no tests were undertaken to determine the complement-fixing ability of the paraproteins, it is of interest that the annular erythema in the case of IgA myeloma reported by Krook & Waldenström (1978) responded to therapy with cyclophosphamide.

The association of pyoderma gangrenosum and paraproteinaemia is considered in Part 2 of this review. Although vessel changes are prominent in this condition, it is unlikely to result simply from a cutaneous vasculitis.

HYPERVISCOSITY SYNDROME

Large amounts of paraproteins in the circulation may present with features of the hyperviscosity syndrome, which is characterized by mucous membrane bleeding, retinopathy and neurological disturbance. Raynaud's phenomenon *per se* is not a feature of the syndrome, unless the paraprotein happens to be cryoprecipitable. The four to five fold increase in relative blood viscosity required before clinical features appear occurs most commonly in Waldenström's macroglobulinaemia where up to 90% of the abnormal protein may be within the intravascular compartment (McGrath & Penny, 1976). Hyperviscosity may also develop in myeloma, particularly with polymeric IgA paraproteins (Roberts-Thompson, Mason & MacLennan, 1976), or with IgG paraproteins with unusual molecular configurations or a tendency to aggregate (Smith, Kochwa & Wasserman, 1965).

It is unlikely that the haemorrhagic phenomena seen in the hyperviscosity syndrome are simply the result of sludging (Russell & Powles, 1978). Antibody activity directed against clotting factors, particularly 5 and 8, platelet dysfunction due to surface coating with immunoglobulin, and non-specific 'absorption' of clotting factors are three of the commoner mechanisms by which paraproteins may interfere with haemostasis (Perkins, MacKenzie & Fudenberg, 1970; Fiddes, Penny & Castaldi, 1971). Purpura may result. Otherwise, cutaneous features are relatively uncommon.

TREATMENT

The foregoing concepts allow a rational approach to the management of patients with paraproteinaemia and vasculitis. Patients with cryoglobulinaemia should be warned against cold exposure, particularly if the serum level is high or the cryoprotein precipitates out at physiological temperature. In extreme cases, it may even be necessary to confine patients to a thermally-regulated environment, since the patient reported by Feizi & Gitlin (1969) developed renal failure after prolonged cold exposure (Cream, personal communication). Patients with Type I or Type II cryoglobulin should be investigated for evidence of a lymphoproliferative disorder and this should be treated accordingly.

Patients with Type II or Type III cryoglobulins should be investigated for evidence of auto-immune disease or chronic infection, particularly hepatitis-B.

Patients with a cutaneous vasculitis may require no specific therapy. Dapsone, however, is a reasonably safe alternative to steroids and has proved particularly useful in cases of cold urticaria and erythema elevatum diutinum (Cream *et al.*, 1971; Fort & Rodman, 1977; Katz *et al.*, 1977). The beneficial effect of dapsone presumably derives from its inhibitor effect on the myeloperoxidase H_2O_2 halide system of the polymorphonuclear leucocyte (Stendhal, Molin & Dahlgren, 1978).

Patients with evidence of systemic vasculitis usually require treatment with steroids and immunosuppressives, alone or in combination. Patients with essential mixed cryoglobulinaemia may benefit from chemotherapy even in the absence of a lymphoproliferative disorder. Neuropathies and other systemic complications have improved with combination therapy using melphelan and prednisolone, but shown no response to prednisolone alone (Ristow *et al.*, 1976). The role of fibrinolytic therapy with phenformin and Orabolin (Dodman *et al.*, 1973) or antiplatelet therapy with aspirin or dipyrimadole (Turpie & Hirsch, 1978) in the treatment of systemic vasculitis is still controversial. An obvious way of removing paraproteins from the circulation is plasmapheresis. It is particularly useful in Waldenström's macroglobulinaemia, and will rapidly relieve features attributable to hyperviscosity (Grey & Kohler, 1973). Plasmapheresis is also an effective method of removing immune complexes from the circulation, but less practical on a long-term basis unless concurrent therapy is capable of preventing further immune complex formation.

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Specific deposits	
B cell cutaneous infiltrates producing an 'M' component	
T cell cutaneous infiltrates plus an 'M' component	Helper cells in Sézary's syndrome. T/B cell interaction?
Light-chain deposition	
Primary and myeloma-associated amyloidosis	Deposition of light chains or immunoglobulin fragments
Quantitative effects of paraprotein production	
Increased susceptibility to infection	Reciprocal depression of other immunoglobulin classes
Hyperviscosity syndrome	Large amounts of circulating paraprotein
Vessel blockage: in cryoglobulinaemia and cold agglutinin disease	Raynaud's and other peripheral vascular changes which result from intravascular mechanisms, depend upon the physical properties of the cryoprotein, and occur independently of complement activation Cold agglutinin disease also reflects the ability of the ability of the cryoprotein to bind red cells
Immunological effects of paraproteins	
Vasculitis	Complement activation by the paraprotein itself, e.g. type I cryoglobulins Complement activation by immune-complexes in which the paraprotein is also the antibody, e.g. type II cryoglobulins Complement activation by immune-complexes with a separate paraprotein (an example of the association between polyclonal B cell stimulation and monoclonal transformation)

British Journal of Dermatology (1980) **103**, 346.

Book Reviews

Methotrexate Therapy of Psoriasis. Effect and Side Effects with Particular Reference to Hepatic Changes. A Survey. ALLAN NYFORS (1980) Thesis, University of Arhus, Denmark. Published by the author, Forskjønnelsen 3, Bergen, N-5000 Norway. Pp. 31. Price £5.00. Also available in *Danish Medical Bulletin*, (1980) **27**, 74–96.

This booklet is a survey based on the author's more than 10 years experience of the use of methotrexate in the management of psoriasis. The author's own clinical experiences are described as well as his basic studies of the light and electron microscopic changes in liver biopsies taken during and after long-term methotrexate therapy for psoriasis.

Much of the information has been published elsewhere but it is a very useful compilation to have all the information collected together. The literature on the subject is also carefully reviewed, as well as the basic pharmacology of methotrexate. Overall, provided due precautions are taken, the results can be considered reassuring.

Histopathologie der Haut. Teil 1: Dermatosen. Edited by U.W.SCHNYDER (1978). Berlin: Springer-Verlag. Pp. 553, B/W illustrations.

It is a great pleasure to see a new rejuvenated phoenix arising in the shape of the two-volumed 'Histopathologie der Haut' edited by Prof. U.W.Schnyder.

Part 1 entitled 'Dermatosen' contains two entirely new and very useful chapters: 'Diseases of Melanin Pigment System' by E. Frank; and 'Skin and Serum Immunofluorescence in Skin Diseases' by S. Jablonska, T.P.Chorzelski and E.H.Beutner. The absence of an account of the value of immunofluorescence in diagnosis and investigation of skin diseases was a notable defect of the previous 1973 edition of this book. One would hope that a third edition might also give more space to ultrastructural studies which are given uneven treatment in this volume.

Most of the chapters, particularly those written by U.W.Schnyder, have been extensively revised with recent pertinent references. The chapter sub-divisions in some instances have been reordered to give a more logical sequence of subject material and several new conditions have been added.

The general layout and type setting shows alterations pleasing to the eye and I have the impression that the quality of the microphotographs has improved technically.

Professor U.W.Schnyder can be heartily congratulated on this venture and the improvements that he has achieved in this important textbook.

E.WILSON JONES

Histopathologie der Haut. Teil 2: Stoffwechselkrankheiten und Tumoren. Edited by U.W. SCHNYDER (1979). Berlin: Springer-Verlag. Pp. 513, 206 B/W illustrations, 1 colour plate. Price DM 260.

'Metabolic Disorders and Tumours' forms the second part of the revised and enlarged edition of the well-known reference work on histopathology of the skin edited by Prof. Schnyder. The original edition of 'Histopathology of the Skin' was published in one volume in 1973 as part of a systematic reference work on special regional pathology.

In the first section of this book Dr Hardmeier has updated his review of metabolic disorders of the skin that appeared in the first edition. The description of both the histological appearances of affected skin and the pathogenetic mechanisms involved are related with conciseness and clarity. All the important metabolic disorders which affect the skin are included and a good overall balance is achieved. The sections on paraproteinaemias and amyloidosis are particularly well written. Electron microscopic and immunofluorescence findings are discussed where relevant and the histological differential diagnosis of most conditions is carefully considered.

Professor Hornstein and Dr Weidner are new contributors and deal expertly with tumours of epithelium, appendages and soft tissues. The quality of illustrations is excellent and the authors have a sensible approach to controversial topics such as intraepidermal epithelioma and so-called metatypical epithelioma.

The section on tumours and hamartomas of blood vessels and lymphatics is capably covered by Prof. Hundeiker. This chapter is again well illustrated, but perhaps the balance of discussion of different disorders is a little uneven in places. The extremely rare condition of angioendotheliomatosis proliferans systematisa receives as much space as Kaposi's sarcoma and more than that devoted to the Stewart-Treves syndrome.

One of the weaknesses of the original edition of *Histopathology of the Skin* was the chapter on cutaneous lymphomas and lymphoproliferative disorders. This section has now been completely re-written by Helmut Kerl and Prof. Kresbach from Graz and the new text is a complete transformation. These writers discuss the difficult topics of skin lymphomas, pseudolymphomas and lymphocytic infiltrates in an expert way with reference to the most up-to-date immunological, histochemical and electron microscopic findings. Although, as one would expect, the Kiel classification of the non-Hodgkin's lymphomas is followed, other classifications such as the long established morphological classification of Rappaport are discussed. Recently recognized conditions such as angioimmunoblastic lymphadenopathy are included and the treatment of the 'histiocytic disorders' is based on modern concepts of the monocyte-macrophage-histiocyte system. The same authors deal with the myeloproliferative diseases and mast cell disorders, this latter group of conditions notable by its absence from the first edition of the book. The quality of photomicrographs in all the sections contributed by Dr Kerl and Prof. Kresbach is excellent and admirably complements the text.

Professor Schnyder and his colleagues should be congratulated on producing an excellent textbook which covers an enormous amount of material in a concise, up-to-date and easy to read way. I sincerely hope that the book achieves the wide circulation it deserves and can unreservedly recommend it to all dermatologists and pathologists who have even a passing interest in cutaneous diseases and their pathogenesis.

NEIL SMITH

A Manual of Dermatology. 2nd ed. DONALD M.PILLSBURY and CHARLES L.HEATON (1980): W. B. Saunders Co. Pp. 360, B/W and colour illustrations. Price £13.00.

This is a 2nd edition of a well known dermatology book which, according to the authors' Preface, is primarily for the medical student and general practitioner.

It is a very well written and published book. It contains a large number of excellent colour and black and white illustrations, although a few of these are poor and do not permit recognition of the condition described. Dermatologists will be able to revise their knowledge of the anatomy and physiology of the skin by reading the excellent first chapter derived from 'A Manual of Cutaneous Medicine' (Pillsbury Shelley & Kligman). They will also find a lot of very interesting information in other parts of the book which is right up-to-date and, for example, discusses the use of 13-cis-retinoic acid.

For the general practitioner it would be helpful as there are sufficient illustrations to act as a mini atlas, and the text is brief and informative. The medical student would also find the book of value, but there are a number of other books available in this country for medical students which are far less expensive.

The authors are to be congratulated on nearly fulfilling the impossible task of writing a small book which will be useful to dermatologists, general practitioners and also medical students.

ROBERT P.WARIN

Skin-irritant and Sensitising Plants found in India, 2nd edn. P.N.Behl and R.M.Captan (1979) New Delhi: S. Chand. Pp. 186.

Most dermatologists are poor botanists and this book is written as a practical guide for Indian clinicians rather than as a dermatological flora. The great advantage of the book is that most of the plants are illustrated. The introduction discusses briefly the chemistry of plant secretions and their skin hazard. The plants are then described in alphabetical order according to their popular names but the vernacular and botanical names are included and are also indexed. The salient features concerning the plants are briefly discussed giving a botanical description, the geographical distribution and their dermatological effect. It is often difficult to be certain whether a plant dermatitis is an allergic or an irritant effect and the authors usually make no such distinction. Occupational hazards are included as are the effect of herbal remedies. This book will be of greatest use not only to Indian dermatologists but also to others for it contains fascinating details about many plants and describes many with irresistable names such as London Rocket, Butter Tree and Biting Pepper.

E.Cronin

Androgenisierungserscheinungen bei der Frau. Edited by J.Hammerstein, U.Lachnit-Fixon, F.Neumann and G.Plewig (1979) Amsterdam: Excerpta Medica. Pp. xi + 306.

This is the report of the proceedings of a symposium held in Berlin in February 1979 on the subject of androgenetic changes in women—acne, seborrhoea, alopecia and hirsutism. The first session considered androgen metabolism in women, the second the clinical syndromes and their endocrinological diagnosis, the third the non-hormonal treatment of acne and androgenetic alopecia and the fourth and longest session, the pharmacology and clinical administration of antiandrogens. A large proportion of the participants were German, and appropriately so, since so many of the pioneering studies of cyproterone acetate have been carried out in Germany. Britain was worthily represented by Profs Cooke and Ebling and four of their colleagues from Sheffield.

Each paper presents a well documented review based on the author's personal experience and on the published literature. Dr Schindler's paper on the possibilities for endocrinological diagnosis in women with androgenetic changes is very informative; not all the investigations he employs form part of the repertoire of all departments of clinical biochemistry but his findings make it clear that greater precision in the diagnosis of these patients is both possible and desirable.

Dr Hammerstein, whose first paper on the use of cyproterone acetate in the androgenetic syndromes was published in 1969, discusses the possibilities and limits of endocrine therapy in these syndromes. Dr Ebling and his colleagues report their studies on the effects of cyproterone acetate on human hair follicles and sebaceous glands. Drs van Wayjen and van den Ende of Amsterdam discuss metabolic effects of cyproterone. Dr Breckwoldt, a clinical endocrinologist from Freiburg, tells of his experience of side-effects provoked by cyproterone acetate with ethinyl oestradiol, in high dosage (100 mg of cyproterone) and low dosage (2 mg of cyproterone). The contraindications to this form of treatment are essentially the same as those for the oral contraceptives.

The symposium concludes with a round-table discussion. This discussion like the papers themselves emphasizes the great benefit that cyproterone can bring to some women with disfiguring syndromes which at present can be reversed in no other way, but also the problems and difficulties that this form of treatment involves especially in its long term maintenance.

Arthur Rook

Fortschritte der praktischen Dermatologie und Venerologie, Band 9. O.Braun-Falco and H.H. Wolff (1979). Berlin: Springer-Verlag. Pp. 396, 69 Abb., 137 tab.X. Price DM. 98.00; approx. US $54.90.

This soft back volume records papers presented at the ninth Progress in Dermatology and Venereology seminar in the University of Munich.

The first took place in 1951 under Alfred Marchionini and was a form of 'Consultants' Course' for the Munich Dermatologists. Such a course has been held every three or four years.

At this last one held in Munich in the summer of 1979, dermatologists attended from nineteen foreign countries, and 800 in all took part.

We are now used to the fact that venereology plays a vital role in the life of a German deramtologist. Articles on extra genital gonorrhoea and Chlamydia infections are of interest.

There are most interesting articles on immunology, photodermatoses and phototherapy, livedo, etc. There is a section on new discoveries, and another on modern therapy.

Whereas 'recent advances' seem to comprise single works and editors' comments, these contributions are more in the nature of review articles with extensive bibliography. The easy expansive style of the papers could well be influenced by the ambience of the Munich Sheraton hotel, where the meeting took place.

M.GARRETTS

Operative Dermatologie Vortrage des 2. Symposiums für Dermatochirurgie Minden-Bad Salzuflen. 26. bis.28. May 1978, K.SALFELD (1979) Berlin: Springer-Verlag. Pp. 265, 155 Ab., 17 Tab.X. Price DM. 92.00; US $50.60.

This imposing soft back book of some 250 pages is based on the summaries of papers presented at a symposium on 'Dermatological Surgery' given at Minden-Bad Salzuflen in May 1978.

The first part concerns discussions on operative surgical techniques, such as wound closure, suture materials, free grafts, full thickness grafts, etc. It is assumed that all dermatological units with teaching facilities are supplied with fully staffed and fully equipped surgical units.

While we in Britain practice the surgery of skin tumours, including biopsies and the excision of small lesions, it is the Continental practice for dermatological surgery to comprise in addition:

(a) surgery of benign and malignant skin lesions;
(b) corrective aesthetic surgery;
(c) surgery of veins.

Ninety percent of the material which follows would be the province of the plastic surgeon in this country, and articles on the position of the flaps and incision lines for various operations take up most of the remaining text.

The surgery of lymphoedema of the penis and scrotum is interesting and details are given for subcutaneous sweat gland resection in the axillae.

This is a useful book to present to your German-speaking plastic surgeon colleague.

M.GARRETT

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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British Journal of Dermatology

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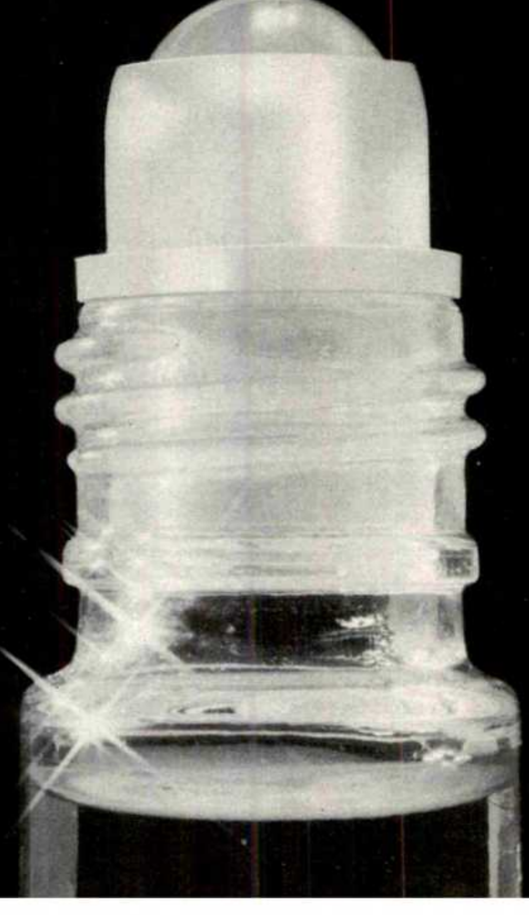

Prescribing Information

PRESENTATION:
Colourless evaporative solution containing 20% w/v aluminium chloride hexahydrate.

INDICATION:
For the topical treatment of hyperhidrosis specifically involving the axillae, hands or feet.

DIRECTIONS FOR USE:
Apply to the affected sites at night as required and allow to dry. Wash off in the morning.

PRECAUTIONS:
Restrict application to affected sites only. Keep away from the eyes.

PACKAGE QUANTITY AND BASIC NHS PRICE:
10ml glass roll-on bottle £2.15.

REFERENCE:
British Medical Journal, 1978, **2**, 84.

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Alphosyl HC

hydrocortisone 0.5% with allantoin and coal tar extract

purpose-designed for psoriasis

*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol 33 No. 4, 106.

sentation A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone, p. 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogenous and is light tan in colour. **s** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage Administration** Apply two to four times daily massaging vigorously into the affected areas.

can cause abnormality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. **Package Quantities** Tubes o 30gm and 45gm. **Basic NHS cost** £1.78 for 30gm, £2.52 for 45gm. **Product Licence No.** 0036/0026.

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Locoid C

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References:

(1) Amer Pract. & Digest of Treat. (1955) 6, 8
(2) Transact. New York Acad of Sci. (1955) 10, 161
(3) Arch. Derm (1972) 106, 335
(4) Rocky Mtn. Med. J. (1955) 52, 6

Prescribing Notes

Dosage & Use. Apply to the affected part two to four times a day, until the infection is resolved. If the underlying condition persists treatment may be continued for up to 14 days and no longer. No more than 60g may be applied during this period. Do not use under occlusion in the presence of infection.

Contra Indications. As with all topical steroids, Locoid C preparations are contraindicated in the presence of viral, tubercular or syphilitic lesions.

Other Precautions. Avoid contact with the eyes. Not to be used in pregnancy, in large amounts or for long periods.
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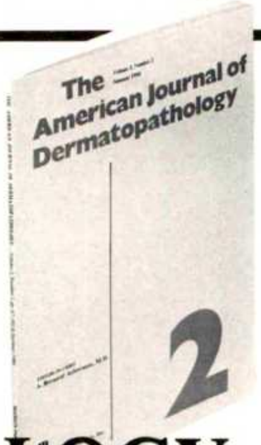

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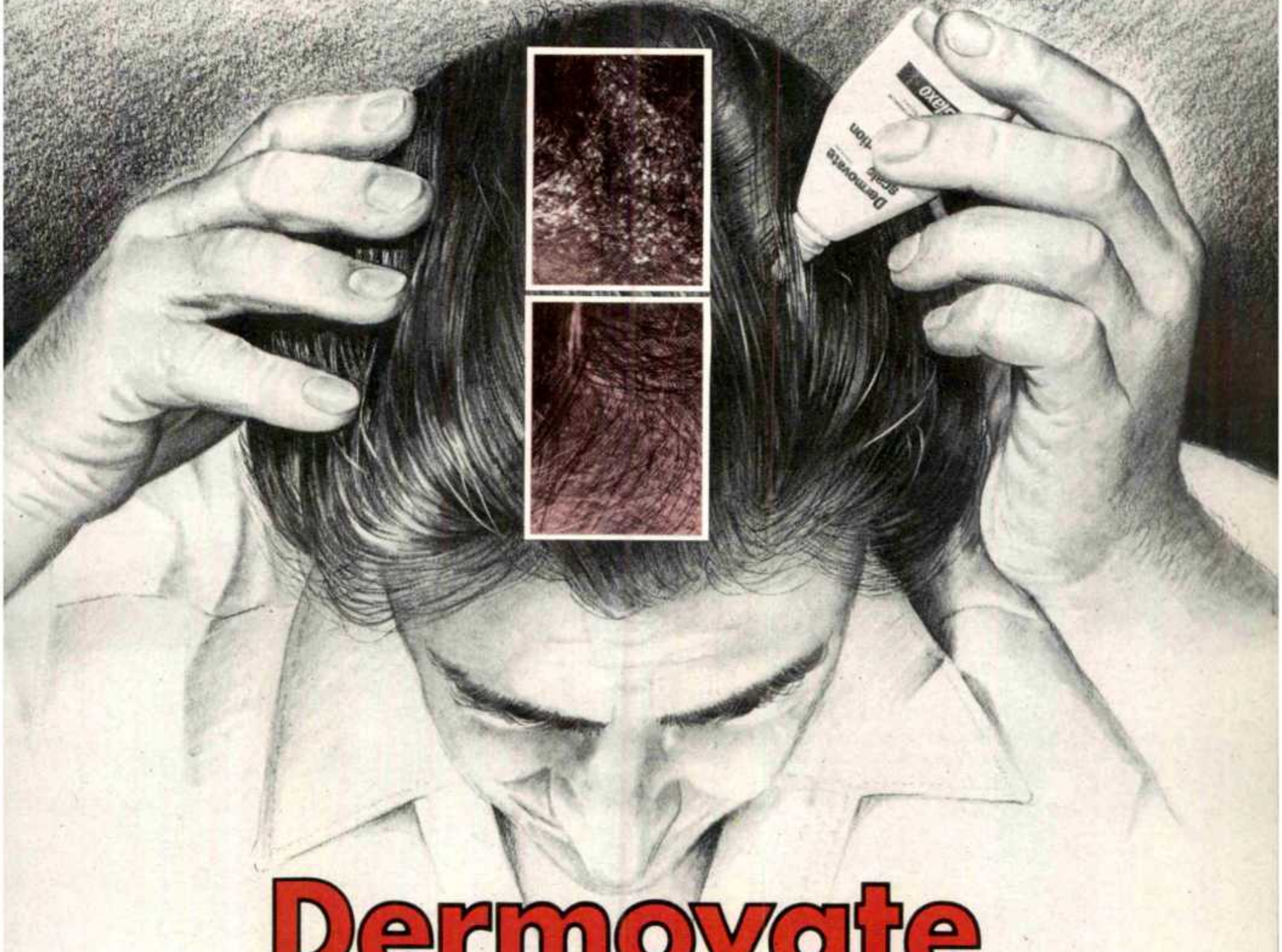

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Presentation

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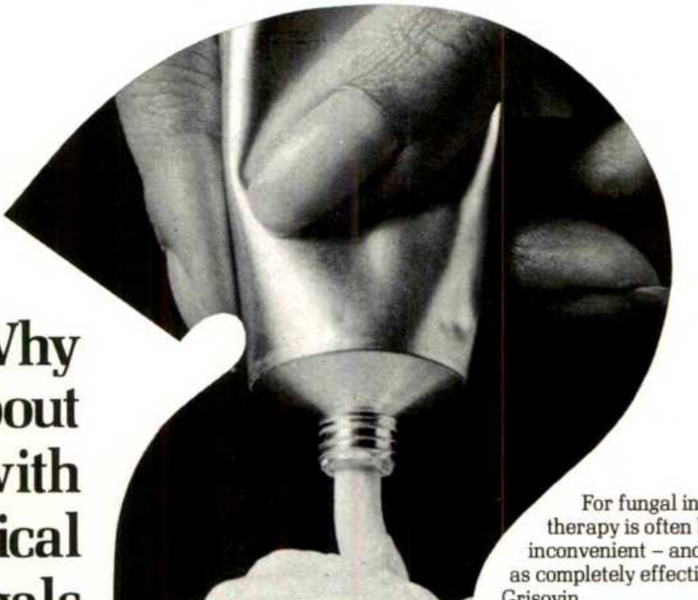

For fungal infections, topical therapy is often both messy and inconvenient – and is unlikely to be as completely effective as oral Grisovin.

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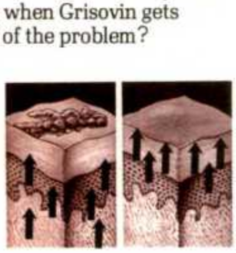

THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.
Children: usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

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Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

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Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

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Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost	**Product Licence numbers**
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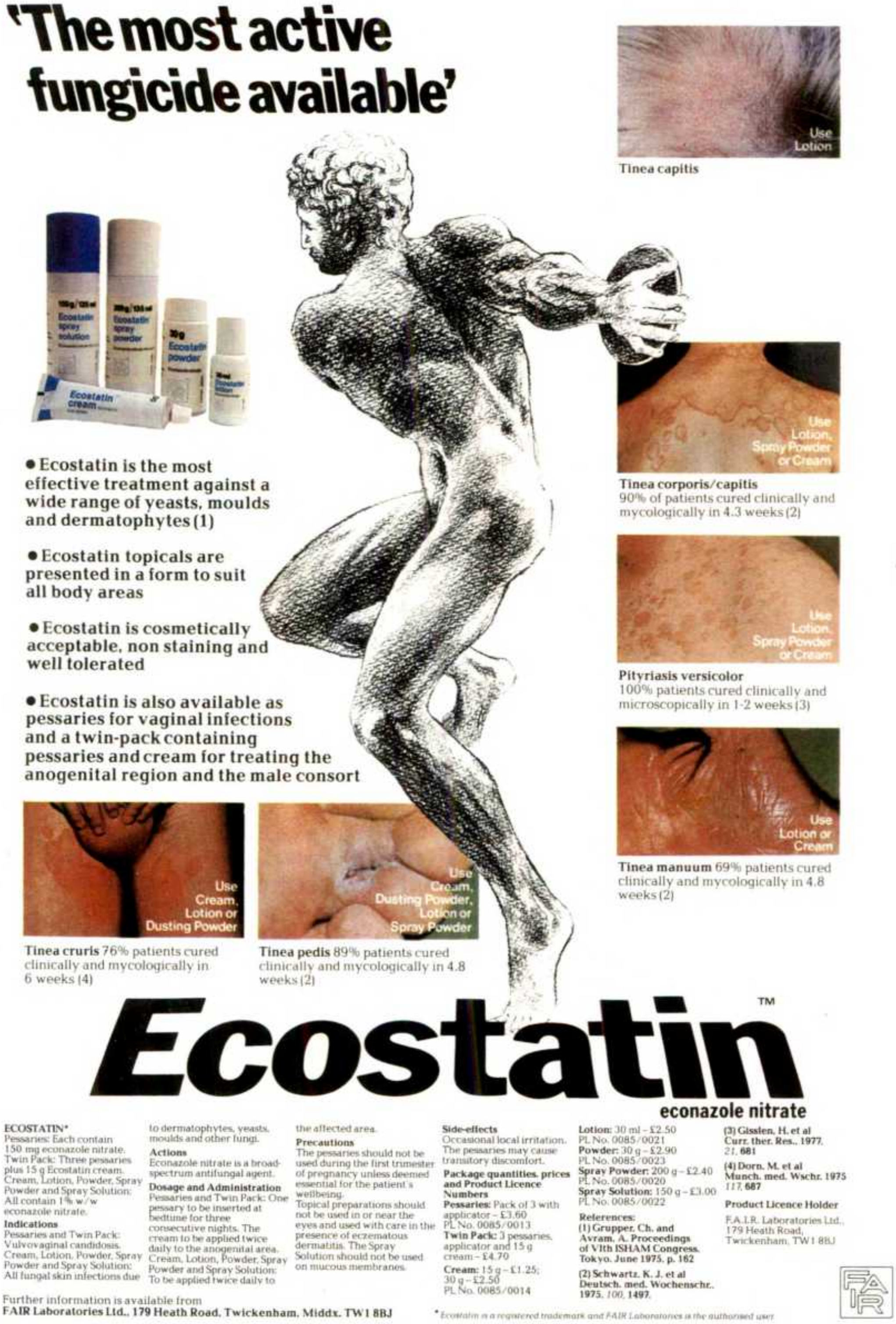
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Econazole nitrate is a broad-spectrum antifungal agent.

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Precautions
The pessaries should not be used during the first trimester of pregnancy unless deemed essential for the patient's wellbeing. Topical preparations should not be used in or near the eyes and used with care in the presence of eczematous dermatitis. The Spray Solution should not be used on mucous membranes.

Side-effects
Occasional local irritation. The pessaries may cause transitory discomfort.

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Cream: 15 g – £1.25; 30 g – £2.50
PL No. 0085/0014
Lotion: 30 ml – £2.50
PL No. 0085/0021
Powder: 30 g – £2.90
PL No. 0085/0023
Spray Powder: 200 g – £2.40
PL No. 0085/0020
Spray Solution: 150 g – £3.00
PL No. 0085/0022

References:
(1) Grupper, Ch. and Avram, A. Proceedings of VIth ISHAM Congress. Tokyo. June 1975. p. 162

(2) Schwartz, K. J. et al Deutsch. med. Wochenschr., 1975. *100,* **1497.**

(3) Gisslen, H. et al Curr. ther. Res., 1977, *21,* **681**

(4) Dorn, M. et al Munch. med. Wschr. 1975 *117,* **687**

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Environmental and Occupational Dermatitis

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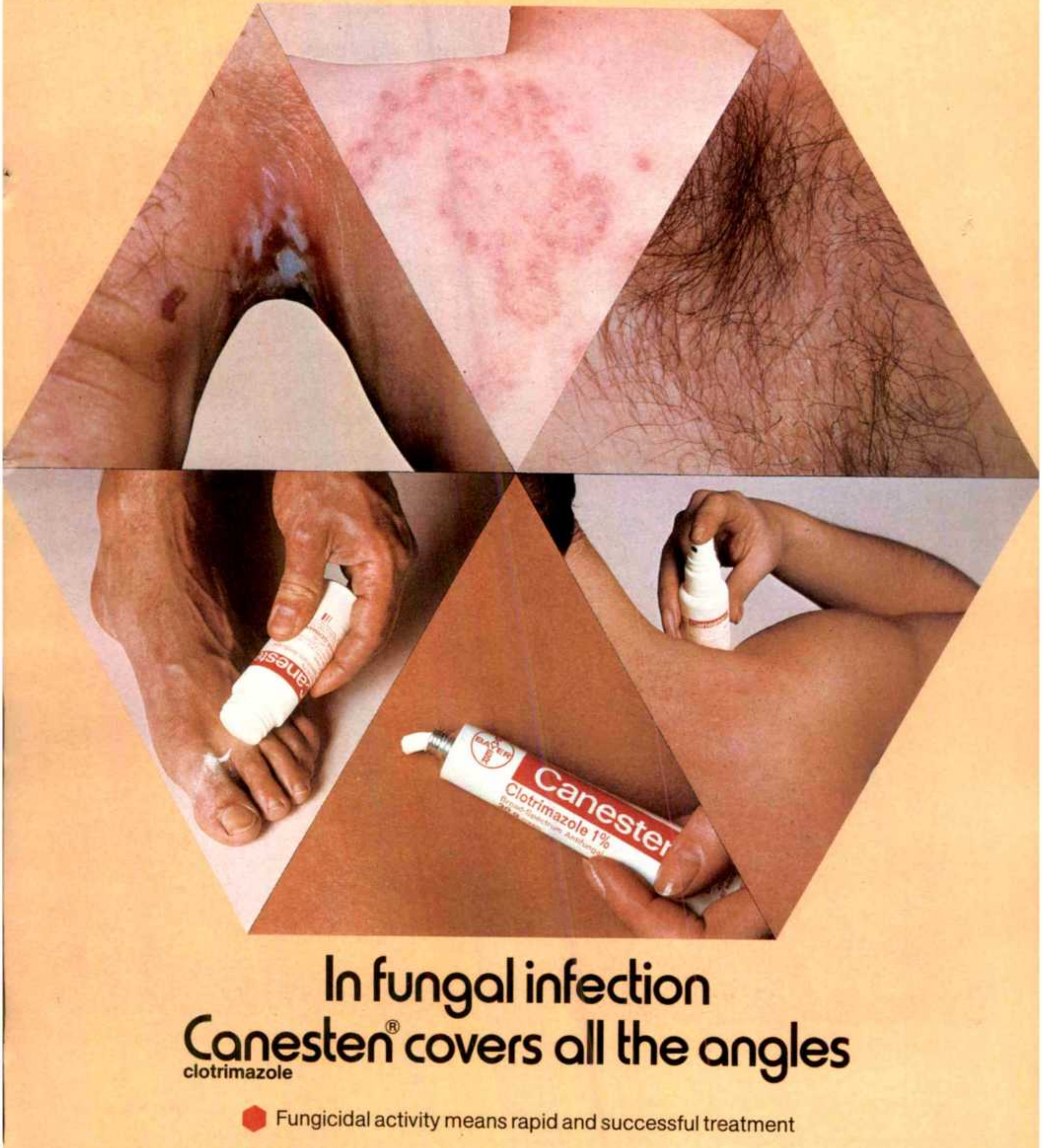

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r Spray Apply to the affected area 2 - 3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic
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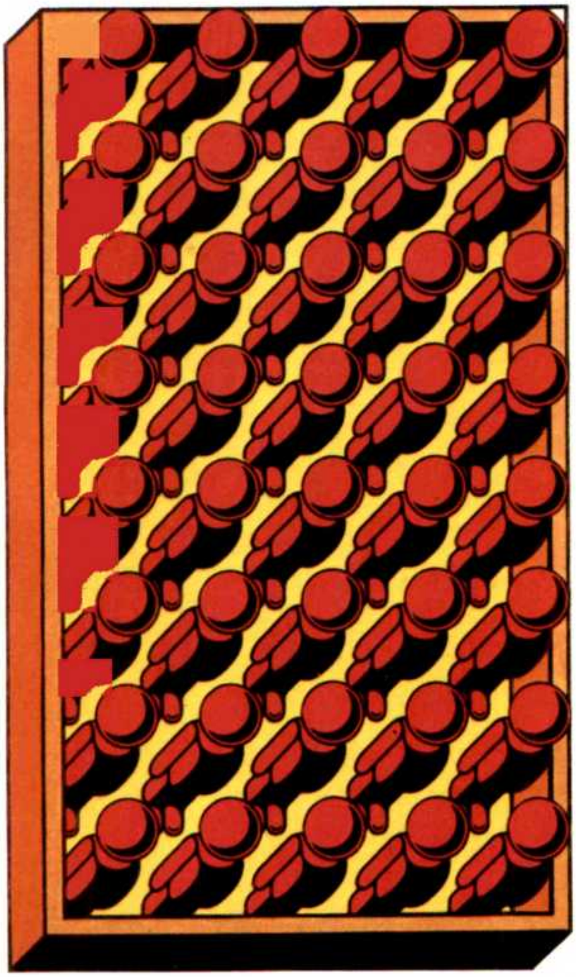
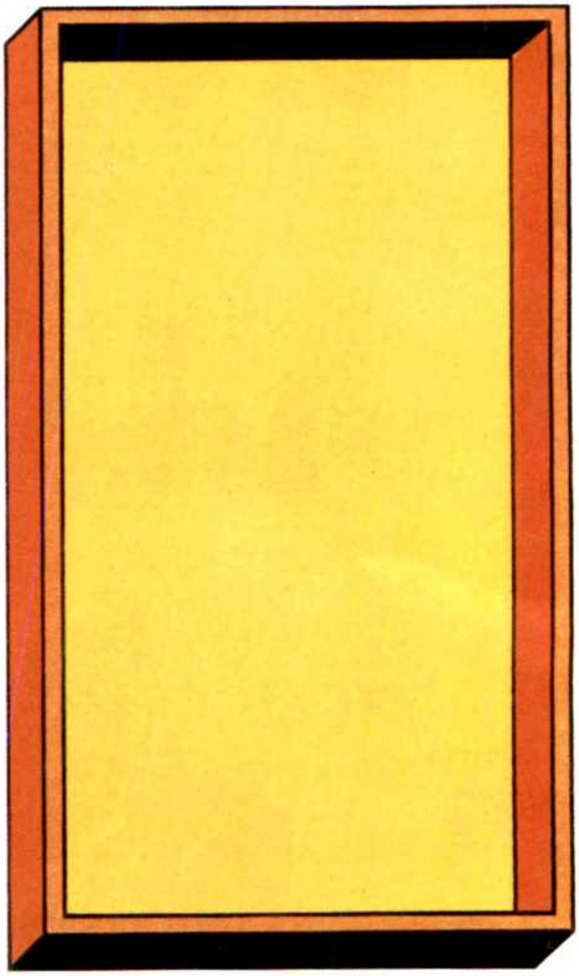


All 40 patients in the trial preferred Dithrocream



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Dithrocream Forte: pale yellow aqueous cream

Indications
For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use
Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications
Acute psoriasis; dithranol sensitivity.

Precautions
Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Basic N.H.S. prices:
Dithrocream 0.1% 50gm tubes £1.81
Dithrocream 0.25% 50gm tubes £1.96
Dithrocream Forte 50gm tubes £2.19

Product Licence Nos.

Dithrocream 0.1%	PL 0173/0029
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1. *British Journal of Dermatology*, 1979, **100**, 475

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Clinical and Laboratory Investigations

Increased chemotactic activity of neutrophil leukocytes in psoriasis

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SUMMARY

The random and active migration of neutrophil leukocytes in agarose was studied in sixty-one patients with psoriasis and in healthy controls. *E. coli* filtrate was used as chemoattractant. The random as well as the active migration was increased in psoriasis. The increase was most pronounced in those cases with widespread psoriasis. In twenty patients who were followed for 1–3 years, the neutrophil chemotactic activity remained essentially unchanged.

Impaired function of neutrophil leukocytes and monocytes with a diminished chemotactic and phagocytic activity has been described in several skin disorders in the past few years (Dahl, 1978). The occurrence of neutrophil leukocytes with elevated activity has, however, been overlooked until quite recently. Increased NBT reduction was observed in psoriasis by Cotterill *et al.* (1974). This increase did not appear to be correlated to disease severity, clinical type or skin area involved and it was not influenced by topical treatment.

In this communication we report an increased chemotactic activity under agarose of neutrophil leukocytes from patients with psoriasis. When the chemotactic activity was followed in the same patient for a long period (1–3 years) it remained essentially unchanged. During the preparation of this paper, similar reports of increased chemotactic activity have appeared using other methods for evaluating chemotactic activity (Whaba *et al.*, 1978, 1979; Langner *et al.*, 1978).

MATERIALS AND METHODS

Patients

There were sixty-one patients (forty-nine men and twelve women aged 22–72 years) with psoriasis varying from mild-moderate stable with only 6–12 nummular lesions (twenty-three patients) to severe often labile with widespread lesions (thirty-one patients). Five patients had moderate to severe psoriasis and two had pustular psoriasis. Most blood samples were drawn before the start of active treatment including potent topical steroids, PUVA baths, and retinoids, but a few patients used U.V. light alone for a long time and occasionally topical corticosteroids.

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Controls

Sixteen healthy subjects (twelve men and four women aged 20–52 years) served as controls.

Preparation of cells

Ten millilitres of blood were drawn into sterile heparinized tubes (143 u heparin/tube), transferred to siliconized tubes and mixed with 10 ml of 2% solution of Dextran 500 in saline (0·9%). The mixture was left at room temperature for about 1 h. The leukocyte-rich plasma-saline mixture was removed, centrifuged for 10 min at 200 g, the plasma-saline pipetted off and the concentration of leukocytes adjusted to 1–1·5 × 10^8 polymorphonuclear leukocytes/ml. Admixture of erythrocytes was avoided by allowing the contaminating erythrocytes to sediment by gravity and by leaving the lowest part of the buffy coat. Smears were made of the white blood cell layer; preparations that were contaminated with red cells were repeated. Usually 90–95% of the cells were neutrophils.

Preparation of agarose plates

The procedure is a modification by Forsgren & Schmeling (1977) of the method described by Nelson, Quie & Simmons (1975). Briefly, agarose plates were prepared containing 1 ml of a 5% solution of agarose, 0·5 ml Hepes buffer solution, 0·5 ml of 10 × 199 Parker's medium (National Bacteriological Laboratory, Stockholm, Sweden) modified with Earle's salts with glutamine but without sodium bicarbonate and 3 ml tri-distilled water.

The solution of agarose (agarose for use in electrophoresis Nr A-6877, Sigma) was made by dissolving the agarose in tri-distilled water on a boiling water bath. Hepes buffer (N-2-hydroxyethyl piperazine-N′-2-ethane sulphonic acid buffer, MW 238, Sigma) 6·2 g was dissolved in 100 ml tri-distilled water. Five millilitres of the mixture were transferred to each plate (tissue culture plastic Petri dish 60 × 15 mm; 3002, Falcon, Oxnard, Calif.) and allowed to harden. The plates were stored inverted for a few hours at 4°C before use. Five series of three wells, each 3 mm in diameter, were made in each plate using a plexiglass template and a punch. Each well held about 10 μl.

Chemotaxis assay

The centre well in each row was filled with leukocytes. The inner wells were filled with 1 × tissue culture medium 199. Three of the outer wells were filled with chemo-attractant consisting of *E. coli* culture (BFE) filtrate (Ward, Lepow & Newman, 1968) and the remaining two outer wells with 1 × tissue culture medium 199. Thus, of the five rows, three were used for active chemotaxis and two for control of random migration. The dishes were incubated for 3 h at 37°C in a humidified atmosphere containing 5% CO_2 in air. The plates were then fixed, stained and the migration quantified as described by Nelson *et al.* (1975). A grid in the microscopic eyepiece was used instead of a microprojector.

Statistics

Student's '*t*'-test was used for calculation of significant differences.

RESULTS

The individual migration distances are shown in Fig. 1 and the mean distances in Fig. 2 and in Table 1. The random as well as the active migration of the leukocytes from the psoriatic patients is increased compared to the migration of healthy controls. The increase is most marked in those with severe psoriasis whereas in those with mild psoriasis only the random migration was significantly increased,

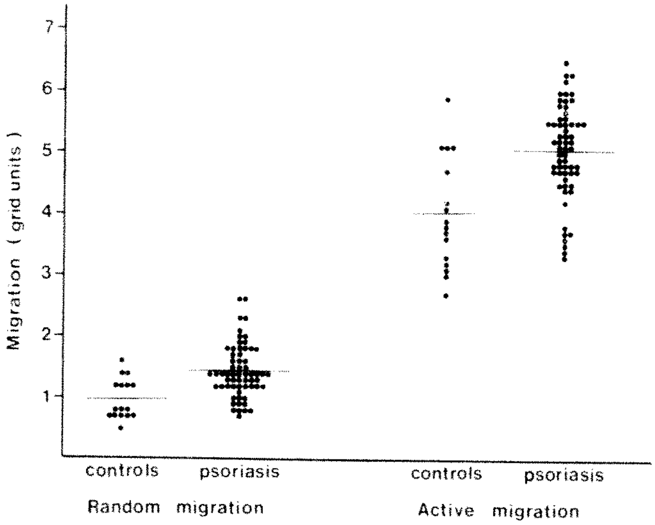

FIGURE 1. Individual migration distances of neutrophil leukcoytes in controls and in patients with psoriasis. Horizontal lines represent means.

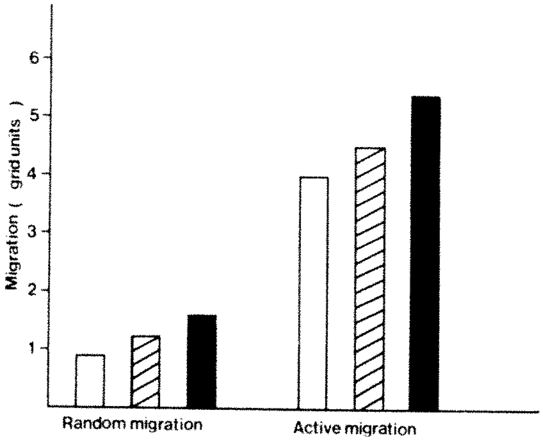

FIGURE 2. Mean migration distances of neutrophil leukocytes in controls and in patients with psoriasis. (Solid columns) severe psoriasis; (hatched columns) mild–moderate psoriasis; (open columns) controls.

although there was a slight increase in the active migration as well. The migration distances were also significantly increased in those with severe psoriasis compared to those with mild psoriasis. Twenty patients have had their chemotactic activity checked several times (2–8) during a period of 2–3 years. The activity remains essentially unchanged, both in those with moderate and those with severe psoriasis. However, in one patient who developed pustular psoriasis, a further increase of his active migration was noted at that time. The influence of various treatments has not been studied systematically. Only five patients on oral retinoid (Ro 10-9359) had their chemotactic activity checked regularly during 3 months. A transient increase of the random migration was observed during the first 2–3 days of treatment.

TABLE 1. Random and active migration of neutrophil leukocytes in patients with psoriasis and in healthy controls

	No.	Random migration (mean ± s.e.)	Active migration (mean ± s.e.)	Degree of significant difference from activity in controls	
				Random	Active
Psoriasis	61	1·43 ± 0·06	5·06 ± 0·76	$P < 0{\cdot}0005$	$P < 0{\cdot}0005$
Psoriasis (severe)	31	1·60 ± 0·08*	5·42 ± 0·11*	$P < 0{\cdot}0005$	$P < 0{\cdot}0005$
Psoriasis (mild–moderate)	23	1·20 ± 0·07*	4·50 ± 0·11*	$P < 0{\cdot}05$	$P < 0{\cdot}10 > 0{\cdot}05$
Controls	16	0·98 ± 0·08	4·03 ± 0·30		n.s.

* Comparison severe vs mild-moderate psoriasis: $P < 0{\cdot}0005$ both for random and active migration.

DISCUSSION

The use of an agarose method for evaluating the chemotactic activity of human leukocytes was described by Nelson *et al.* (1975). It has been critically evaluated in several recent papers (Repo, 1977a, b; Orr & Ward, 1978) and found to be simple and inexpensive and to give reproducible results. The reproducibility of the modification reported here is comparable to that of the Nelson method. Other advantages are that no microscope filter membranes are used and fewer cells are needed.

Several reports in recent years have demonstrated that psoriatic scales—even when bacterial contaminants have been inactivated—have chemo-attractant properties (Langhoff & Müller, 1966; Tagami & Ofuji, 1977a, b; Lazarus, Yost & Thomas, 1977; Dahl, Lindroos & Nelson, 1978) and it has been concluded that this is an important contributory factor to the psoriatic process.

This study on the chemotactic activity of neutrophil leukocytes reveals a marked increase in both directed and random migration in patients with psoriasis compared to healthy controls. The increased response is most marked in those with severe, widespread and active psoriasis, whereas in those with a more stable and moderate psoriasis, it is less pronounced. The patterns of activity tend to remain essentially unchanged over long periods. Although no systematic studies have been conducted on the activity during various types of treatment, it is evident that in severe psoriasis the activity is not 'normalized' although it occasionally decreases during periods of transient improvement.

Various modifications of the Boyden technique for chemotaxis have been used in three other recent reports on neutrophil chemotaxis in psoriasis (Whaba *et al.*, 1978, 1979; Langner *et al.*, 1978) and also in a survey of inflammatory and immune cell function in psoriasis (Krueger, Hill & Jederberg, 1978a). In the first report by Whaba *et al.* (1978), a mixture of serum and *Shigella flexneri* lipo-polysaccharide heated at 56°C was used as chemoattractant to study neutrophil chemotaxis in twenty-two patients with psoriasis. In all but two, the response was higher than in the controls and the mean response was more than 100% higher. Their results differ in two respects from ours: they found no increase in spontaneous migration and no difference in chemotactic response in severe versus minor psoriasis. The explanation for this might possibly be due to differences in the methods and also to the small number of patients compared (seven versus five patients). However, in a second report by Whaba *et al.* (1979), the results of their first study were confirmed and expanded. Furthermore they found normal chemotactic activity in psoriatic patients treated with diphylline.

Langner *et al.* (1978) reported—in a preliminary study on forty patients with psoriasis—that those with active disease had an increased neutrophil response to casein, whereas those with stable or inactive psoriasis showed a normal response. These results are in agreement with those presented here on differences in response between those with severe and those with stable psoriasis.

Langner *et al.* (1978) also compared the response to casein with that of epidermis, psoriatic scales, blister fluid and psoriatic serum and found a highly increased response especially to serum. Whaba *et al.* (1979) in their second report on neutrophils claim that undiluted plasma from patients with widespread psoriasis has chemoattractant properties on normal human leukocytes whereas plasma from those with mild psoriasis and from healthy controls lacks this property. On the other hand they found that preincubation of normal leukocytes with psoriatic plasma diminished the chemotactic activity.

In view of these independent reports of increased neutrophil chemotactic response, the findings of Krueger *et al.* (1978a) that chemotaxis appeared normal in fourteen patients with psoriasis seems puzzling. Further they found no changes in phagocytosis or in NBT reduction. They were not able to confirm the increased NBT reduction by neutrophil leukocytes in psoriasis reported earlier by Cotterill *et al.* (1974). On the other hand Kreuger *et al.* (1978b) reported a strongly increased monocyte chemotaxis as well as monocyte NBT reduction. The increased NBT reduction was more marked in those with active disease. Later, Bar-Eli *et al.* (1979) reported that psoriatic monocytes have an increased phagocytic as well as bactericidal activity. Monocytes also seem to play a major role in the fibrin crystallization (microclot formation) observed in the blood of psoriatic patients after addition of various endotoxins (Juhlin & Shelley, 1977; Shelley & Juhlin, 1977).

There is now ample evidence that both neutrophils and monocytes behave differently in psoriasis. These changes can thus be added to the list of several other non-epidermal aberrations in psoriasis such as decreased lymphocyte response (Guilhou *et al.*, 1976), and decreased reactions to intradermal streptokinase-streptodornase and to challenge with DNCB (Kreuger, Jederberg & Ogden, 1976) as well as to intradermal kallikrein (Michaëlsson, 1970).

ACKNOWLEDGMENTS

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An assessment of factors influencing flexibility of human fingernails

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SUMMARY

A new instrument has been developed and used to determine the effect of various materials on nail flexibility. It repeatedly flexes longitudinal nail sections through 90 degrees and records the number of flexions required to fracture each section.

Immersion in water or a phospholipid–water preparation (PLW) greatly increases the flexibility of untreated and lipid extracted nails; immersion in mineral oil does not.

Nail flexibility is directly related to the duration of their immersion in water. During water immersion, nail weight increases by 22% of its original weight within 2 h, and then decreases. The rapid increase in nail flexibility during water immersion is related to nail water content.

It is possible to prolong the flexibility of previously hydrated nails by the application of PLW or mineral oil. PLW is more effective than water alone in prolonging flexibility of nails extracted with a mixture of acetone, water and acetic acid.

The physical properties of nails are commonly described in terms of 'brittleness', 'hardness', or 'weakness', usually ill-defined subjective assessments. In an attempt to quantify certain physical properties of nails, Baden (1970) described techniques for measuring their elasticity, water-binding capacity and the rate at which water diffuses through them. He demonstrated that water content of nail plate is directly related to relative humidity and that prior lipid extraction did not affect water uptake or rate of water loss. Maloney & Paquette (1977) described equipment for performing flexural, tensile, tearing and impact absorption tests on nails and demonstrated differences related to age, sex and digit of nail origin. Newman & Young (1967) reviewed and strongly criticized a method of

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Part of this work was presented in poster form at the Society for Investigative Dermatology Annual Meeting, 7–9 May, 1979 (*Journal of Investigative Dermatology* (1979) **72**, 205).

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assessing fingernail hardness by the Knoop Indenter, an instrument which measures hardness by measuring depth of indentation of a fixed load on a constant area.

We have devised a new instrument, the 'nail flexometer', to measure flexural properties of nails. This makes possible the study of the role of different components in maintaining flexibility and allows objective assessment of effects of topically applied preparations. To date, there is little published evidence that topically applied preparations alter the physical properties of nails. Dixon (1967), in fact, found that a commercial nail 'hardener' had no beneficial effect on altering nail splitting.

MATERIALS AND METHODS

The nail flexometer

The nail flexometer (Figs 1 and 2) flexes a longitudinal nail section through 90 degrees back and forth, and records the number of flexions required to fracture the nail section (fracture number = FN). The nail section is held proximally between two brass plates, bevelled with a 1·4 mm radius of curvature on a steel shaft which is moved back and forth by an electrically driven cam rotating at 50 r.p.m. The protruding distal end of the nail is bent through 90 degrees in each direction as it sweeps past a plastic wheel. A metal flange attached to the wheel thereby reguarly interrupts a light beam. When the nail breaks, it can no longer move the wheel and the light beam is no longer interrupted. A time delay system then stops the machine and the number of nail flexions is recorded.

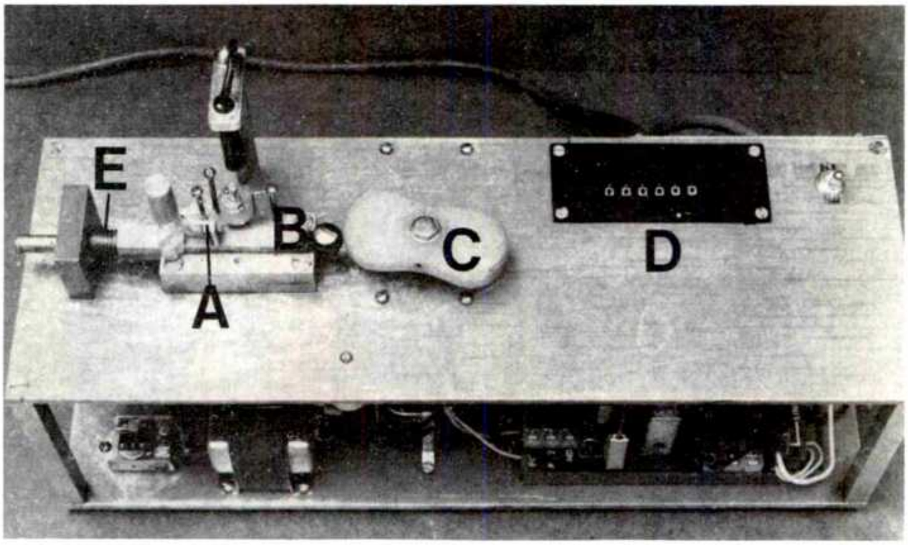

FIGURE 1. Nail flexometer, general view. (A) Nail. (B) Steel shaft. (C) Rotating cam. (D) Number of nail flexions. (E) Spring.

Nail specimens

Human cadaver fingernails cut as shown in Fig. 3 were used. Paired sections from the same nails were used in all experiments for comparative purposes, or one section served as a control. Nail sections were marked distally for orientation. As many as ten pairs of nail sections were tested in each experiment. In order to minimize variation among different nails in later experiments, nails were used from only one specific finger position, e.g. all middle fingernails (Tables 1 and 3). Following

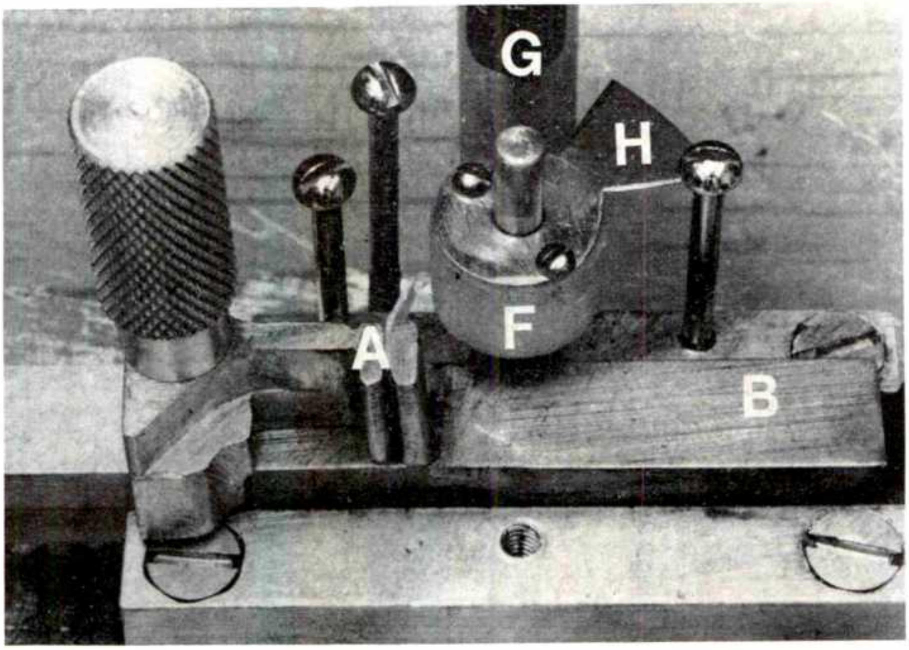

FIGURE 2. Nail flexometer, close-up view. (A) Nail held between bevelled brass plates. (B) Steel shaft. (F) Plastic wheel. (G) Light beam intermittently interrupted by metal flange. (H) Metal flange.

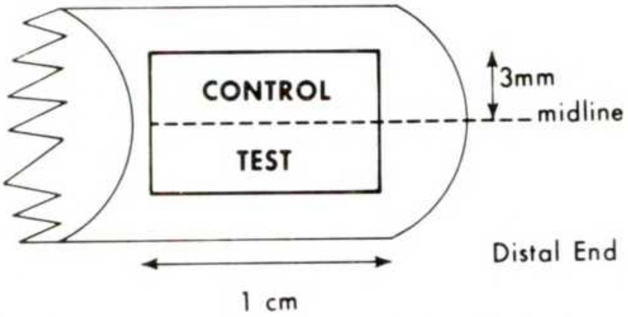

FIGURE 3. Diagram of cadaver nail, showing nail sections used.

immersion of nail sections in water or one of the study preparations, the surfaces were immediately wiped of excess solution with a paper towel.

Topical agents tested

Nail sections were treated with distilled water, mineral oil (purchased from Pharmacare, Inc.) or a phospholipid–water preparation (PLW) containing 22% phosphatides in a proprietary vehicle (supplied by Key Pharmaceuticals, Inc.). The PLW is a water–alcohol dispersion of phosphatides; the exact physical state of the dispersion was not determined. A similar preparation (PLW-C) from which the phosphatides were omitted was used as a control. The commercial nail preparation tested was Sally Hansen 'Hard as Nails'. All experiments were performed at ambient temperature (20°C).

TABLE 1. Direct comparison of mineral oil and PLW on paired nail sections

Time (h)	Finger position of nails	Number of pairs of nail sections studied	Mineral oil Mean FN (s.d.)	PLW Mean FN (s.d.)	Significance
1	Index	9	1·8 (1·4)	34·2 (29·7)	$P<0·01$
6	Little	10	1·8 (0·6)	44·6 (26·4)	$P<0·0004$
24	Ring	10	3·3 (5·1)	44·4 (18·1)	$P<0·0001$

TABLE 2. Effect of PLW or mineral oil on previously hydrated nails

Time (h)	Number of pairs of nail sections studied	Treated nails (previously hydrated) Mean FN (s.d.) x	Paired controls (previously hydrated) Mean FN (s.d.) y	x/y	Significance
PLW treated nails					
1	10	31·3 (15·9)	12·4 (10·7)	2·5	$P<0·005$
3	9	52·9 (25·1)	9·0 (7·0)	5·7	$P<0·002$
6	10	23·2 (16·8)	7·7 (11·1)	3·0	$P<0·002$
18	10	28·7 (17·0)	22·8 (13·8)	1·3	$P<0·33$
Mineral oil treated nails					
1	9	24·0 (8·9)	16·4 (13·0)	1·5	$P<0·02$
3	10	69·9 (70·0)	30·0 (27·9)	2·3	$P<0·03$
6	6	29·0 (15·6)	2·3 (1·6)	12·6	$P<0·01$
18	9	70·3 (40·3)	32·3 (19·1)	2·2	$P<0·02$

Procedure

The effect of water immersion on nail flexibility was measured. Nail sections were immersed in water for periods of 0·5, 1, 2 or 21 h, blotted dry and immediately studied with the nail flexometer. Paired sections of the same nails not immersed in water served as controls. Ten other nail sections were weighed, immersed in water, blotted dry and reweighed at 0·5, 1, 2, 4, 6 and 24 h.

The effects of immersion of nails in mineral oil or in PLW were studied by treating paired sections. The FN was determined at 1, 6 and 24 h.

To study the effects of various topical preparations in prolonging the flexibility of hydrated nails, sections of test and control nails were immersed in water for 2 h. The test specimens were then immediately painted with mineral oil or PLW and the controls left in air, unpainted. The FN was determined at 1, 3, 6 or 18 h following water immersion.

Paired nail sections were placed in a solution of 90% acetone, 9% water and 1% acetic acid for 1 h to extract phospholipids (Fleischer & Fleischer, 1967). This relatively mild extraction procedure was designed to remove lipid extractable material, including surface oils, without structural modification of nail proteins. These lipid extracted nails were left in air for 18 h at 20°C and then one section from each pair was immersed for 1 h in water, mineral oil or PLW before being tested; the paired untreated lipid extracted sections acted as controls.

Lipid extracted, paired nail sections were placed in water or PLW for 1 h and then left in air for 3 h before testing. In another experiment, lipid extracted paired nail sections were placed in PLW or PLW-C and then left in air for 3 h before testing.

Untreated nail sections were painted on their superior surface three times with a commercial nail 'hardener', allowing the hardener to dry between coats. Eighteen hours later, the FN of treated and paired untreated sections was determined.

Statistical analysis

The FN of the treated nail sections and their paired controls were compared using the analysis of variance block design technique. In seventeen of twenty-two block comparisons, there was an acceptable lack of variability ($P>0{\cdot}05$) of the standard deviation of the mean of individual pairs.

In the other five block comparisons there was wider variation in the flexibility of the nails tested, but in each of these groups the flexibility of only one or two nails was significantly different from that of the others, and the difference between the flexibility of test and control groups remained significant in spite of the variation of nails tested. The five exceptions were the previously hydrated 6 h PLW treated, and the 1 and 3 h mineral oil treated nail sections (Table 2), the lipid extracted nail sections treated with water (Table 3) and the nail sections treated with PLW-C.

The nail weight changes during water immersion were compared using the paired t-test.

TABLE 3. Lipid extracted paired nail sections immediately after immersion in test compound for 1 h

Test compound	Finger position of nails	Number of pairs of nail sections studied	Test nails Mean FN (s.d.)	Paired controls Mean FN (s.d.)	Significance
Water	Little	10	39·5 (19·9)	12·1 (15·7)	$P<0{\cdot}0002$
PLW	Middle	9	42·0 (27·0)	2·4 (1·8)	$P<0{\cdot}002$
Mineral oil	Index	10	12·5 (9·6)	9·7 (7·9)	$P<0{\cdot}58$

RESULTS

The effects of water immersion on nail flexibility are given in Table 4 and Fig. 4. Water immersion resulted in a significant increase in FN. After 1 h, the mean FN was 3·5 times that of controls ($P<0{\cdot}005$) and by 21 h, 8·8 times that of controls ($P<0{\cdot}001$).

The effects of water immersion on nail weight are shown in Fig. 5. Weight increases by 18·3% within one half-hour and continues to increase during the next 1·5 h. The maximum gain (22·3%) is at 2 h, but further immersion results at 24 h in a slight decrease in weight to 21·8% above the original weight ($P<0{\cdot}05$).

The comparative effects of mineral oil and PLW on previously untreated paired nail sections are given in Table 1. The FN of the mineral oil treated nails remained low even after 24 h immersion. The FN of the PLW treated nails was much greater than the mineral oil treated nails at 1 h (PLW FN = 34·2, mineral oil FN = 1·8, $P<0{\cdot}01$). There was a further increase in the FN of PLW treated nails by 6 h, but no further significant change at 24 h.

When applied to hydrated nails, PLW was effective in maintaining increased flexibility up to 6 h, but not at 18 h (Table 2, Fig. 6). Application of mineral oil was effective in maintaining increased flexibility of previously hydrated nails up to 18 hours (Table 2, Fig. 6).

TABLE 4. Effect of water immersion on nail flexibility

Time (h)	Number of pairs of nail sections studied	Water immersed nails Mean FN (s.d.) x	Untreated paired controls Mean FN (s.d.) y	x/y	Significance
0	10	6·3 (5·1)	6·6 (5·1)	0·95	$P<0{\cdot}89$
0·5	6	70·5 (50·6)	31·2 (14·4)	2·3	$P<0{\cdot}06$
1	7	54·4 (32·6)	15·6 (9·9)	3·5	$P<0{\cdot}005$
2	9	33·6 (22·6)	14·1 (12·4)	2·4	$P<0{\cdot}05$
21	8	33·6 (16·0)	3·8 (3·6)	8·8	$P<0{\cdot}001$

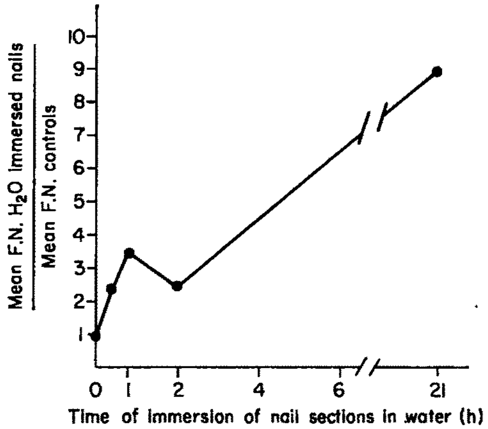

FIGURE 4. Effect of water immersion on nail flexibility.

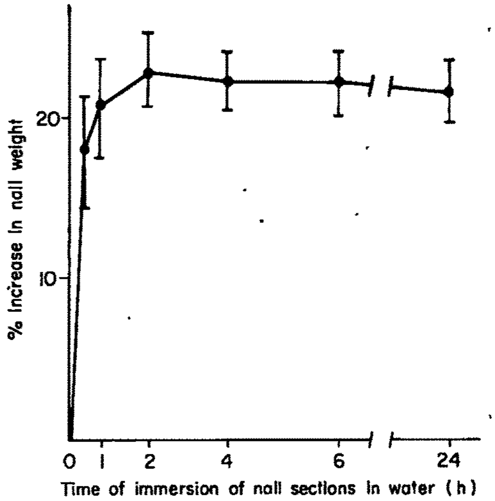

FIGURE 5. Effect of water immersion on nail weight. Ten nail sections were tested. Finger position = middle. There was a significant difference ($P<0{\cdot}05$) between the following pairs of results: 0–0·5, 0·5–1, 1–2, 2–24, 6–24 h. Bars represent 2 × s.d.

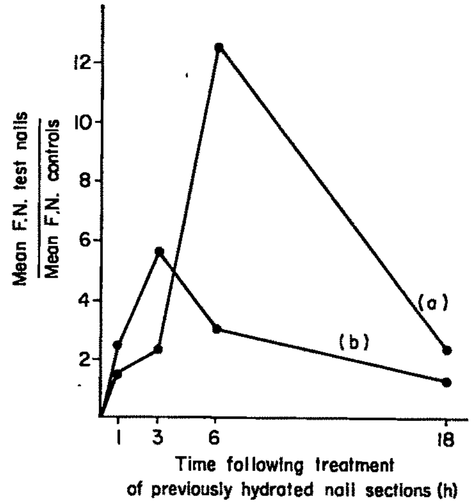

FIGURE 6. Effect of (a) mineral oil and (b) PLW on previously hydrated nails.

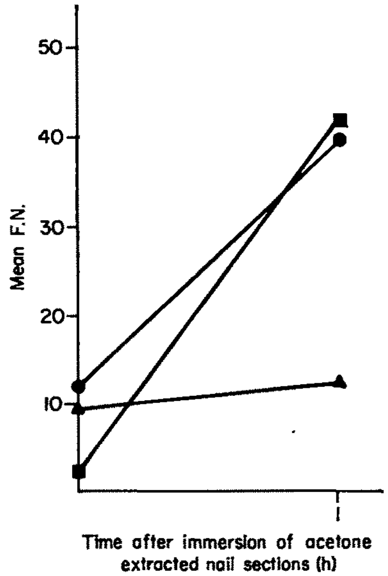

FIGURE 7. Effect of different compounds on lipid extracted nails .(●) Water; (■) PLW; (▲) mineral oil.

The immersion of lipid extracted nails for 1 h in either water ($P<0.0002$) or PLW ($P<0.002$) increased the FN of nail sections (Table 3, Fig. 7), but immersion in mineral oil did not.

Three hours following immersion of paired lipid extracted nails for 1 h in water or PLW, sections of PLW treated nails remained more flexible than those treated with water (water mean FN=3·8, PLW mean FN=8·6, $P<0.03$. Number of pairs tested=10). Three hours following immersion of paired lipid extracted nails for 1 h in PLW or PLW-C, the PLW nails remained more flexible than those treated with PLW-C (PLW mean FN=30·1, PLW-C mean FN=19·0, $P<0.05$. Number pairs tested=10).

The FN of untreated control sections (Table 4) and lipid extracted control sections (Table 3) were low; in both cases, the nails fractured after few flexions.

Following the application of three coats of a commercial nail hardener, there was no significant difference in their FN's from those of paired untreated nail sections (Hard as Nails mean FN = 23·9, paired controls mean FN = 23·3, $P<0{\cdot}91$. Number of pairs tested = 10).

DISCUSSION

The nail flexometer has been used to measure changes in physical properties of fingernails caused by various agents. The property measured by the nail flexometer is the ability of a nail section to withstand repeated flexions. The weakening of the nail leading finally to fracture may be analogous to the phenomenon of 'metal fatigue'.

The human nail plate shows an X-ray diffraction pattern which indicates that the alpha-keratin proteins are laid down in an ordered matrix (Baden, 1970). The long axis of the filaments is orientated perpendicularly to the direction of growth. When tested in the nail flexometer, the nail fracture is parallel to the long axis of the alpha-keratin filaments.

There is considerable variation in the flexibility of nails of different subjects and nails from different fingers of the same subject. This problem has been dealt with by statistical methods. Table 4 (Time '0') shows the FN's for untreated paired nail sections and there is no difference in the results of paired sections. For each nail section tested the paired nail section was, therefore, used as a control.

Effect of water

After immersion in water for 1 h, the weight of nails is increased by 21%; nail flexibility is also increased significantly. The degree of nail hydration is, therefore, a major factor in determining nail flexibility. This may be because water molecules hydrate the alpha-helical proteins and allow them to move more freely with respect to each other.

It may be expected that nails, in many respects, would behave in a fashion similar to that of stratum corneum and, indeed, the elastic modulus of stratum corneum is a function of water content (Van Duzee, 1978).

After immersion in water for more than 2 h, flexibility continues to increase, but nail weight slowly decreases. This was an unexpected finding for which we have no explanation.

Effect of mineral oil

Mineral oil treated nails show no increase in flexibility, but when mineral oil is applied to previously hydrated, very flexible nails, their flexibility is prolonged. This may result from an occlusive effect of mineral oil which would delay the evaporation of water, but the exact explanation is not known.

Effect of phospholipids

Membrane phospholipids strongly bind water and may thereby contribute to nail flexibility (Keith, Snipes & Chapman, 1977). Immersion of nails in PLW, containing 22% phospholipids, greatly increases nail flexibility, but this effect may be due almost entirely to the effect of the water in the PLW. Indeed, water and PLW both increase the flexibility of lipid extracted nails to a similar extent.

Water evaporates rapidly from nails (Baden, 1970) and the addition of phospholipids may prolong flexibility by binding water molecules. Previously hydrated nails coated with PLW maintained increased flexibility for up to 6 h and lipid-extracted nails which had been immersed in PLW retained greater flexibility at 3 h than those treated with water alone.

The final experiment with the PLW-C confirmed that the ability to maintain flexibility was related to the phospholipids rather than to the other components of PLW.

It is possible that clinically 'brittle' or 'fragile' nails fracture more easily and are less able to withstand repeated stress than normal nails. We have not yet tested nails from patients with this problem.

A logical approach to reducing 'brittleness' would be to enhance hydration of nails by using approaches such as the addition of phospholipids, which we have demonstrated to be effective in increasing and maintaining increased flexibility.

From a practical standpoint, subjects are concerned that their nails are attractive by virtue of being able to resist chipping, tearing, breaking and splitting. Available products designed for nail care are primarily lacquers or polishes which may enhance nail hardness and strength but probably not flexibility. The PLW treatment is designed to impart greater flexibility through greater water binding of the nail.

We could detect no increase or decrease in flexibility of nail sections treated with a widely used commercial 'nail hardener'.

In conclusion, we have described a new machine to assess nail flexibility *in vitro*, and have demonstrated changes in nail flexibility caused by water, mineral oil and a phospholipid preparation.

ACKNOWLEDGMENTS

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In vitro demonstration of specific immunological hypersensitivity to scabies mite

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SUMMARY

An extract prepared from 200 adult female scabies mites was used for prick and intracutaneous testing in twelve patients with previous scabies infestation, as well as in six healthy controls and three persons with skin sensitivity to *Dermatophagoides pteronyssinus* who had never had scabies before. Seven individuals who had had scabies less than a year prior to the testing had positive intracutaneous (immediate type) reactions, whereas all the five who had had scabies more than a year before had negative reactions. The prick tests were negative in all cases. No skin reactions could be evoked in the controls. The passive transfer, or Prausnitz-Küstner, test was positive with the scabies mite extract and serum from three of five patients tested on one healthy individual, and with both of two sera tested on a second healthy individual. These findings indicate that immediate type hypersensitivity reactions may occur with scabies infection.

Several investigations have been performed in patients with scabies in order to demonstrate and identify immune reactions against scabies mite antigens. Skin testing has been the usual method (Sézary, Ducourtioux & Felsenswalb, 1931; Glaouberson, Trakhtenberg & Blindman, 1940; Berna, 1942; Mellanby, 1944; Heilesen, 1946). Allergen extracts employed for this purpose have been crude heterogeneous mixtures containing unknown contaminating components such as blood proteins, microbes and epithelial debris, which may be responsible for erroneous results (Bourguignon, 1846; 1852; Gerlach, 1857; Kitchevatz & Pochtitch, 1934).

This paper presents results of investigations with an extract made solely of isolated scabies mites on patients who previously had suffered from scabies, using the prick and intracutaneous methods. Attempts to demonstrate reaginic antibodies to the scabies mite extract by means of the Prausnitz-Küstner (P.K.) test are also presented.

MATERIALS AND METHODS

Individuals

Twelve persons (five women, seven men) between the ages of 15 and 61 years (average 26·3), who had

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been successfully treated for scabies between 6 weeks and 2 years prior to the investigation, were skin tested. Skin tests were carried out after the skin eruptions had subsided in order to avoid non-specific reactions.

Six healthy employees at the Department of Dermatology who had never suffered from scabies or atopic disease, and three persons with skin sensitivity to Dermatophagoides pteronyssinus (DP) who had never had scabies, served as controls.

Sera

Sera from all individuals were stored at −20°C and were thawed immediately before use. The sera used for injections were negative for syphilis and hepatitis virus B infection.

Extract of scabies mite

Two hundred adult female scabies mites were collected from patients with scabies, in the course of 2 months, by removing the mites undamaged from the burrows into a clean glass cell where they were stored at a temperature of −20°C. The mites (0·8 mg) were crushed and extracted for 12 h with a solution consisting of 0·9% NaCl, 0·5% phenol, and 0·01% EDTA (ethylenediaminetetraacetic acid) in distilled water up to 2 ml. The mite extract was filtered through a Sartorius membrane filter, SM 11107, 0·2 μm, under sterile conditions in a laminar air flow bench. The concentration of the final extract was estimated to be 1:2500 (weight/volume). The extract was kept at +4°C until used 1–2 weeks later.

Extract of Dematophagoides pteronyssinus

This was the commercially available aqueous extract obtained from ALK (Allergologisk Laboratorium, Copenhagen). The concentration used for prick testing was 1:100 and for intracutaneous testing 1:1000 (w/v), respectively.

Serum IgE

The IgE concentrations in serum were determined using the Phadebas PRIST test (Pharmacia AB, Uppsala, Sweden). The upper limit of normal IgE value was considered to be 122 u/ml in adults (Nyco, Oslo, Norway).

Radioallergosorbent test (RAST)

The Phadebas RAST test (Pharmacia AB, Uppsala, Sweden) was used to quantify circulating IgE antibodies to DP. The RAST results were expressed in Phadebas RAST Classes 0, 1, 2, 3 and 4, according to a reference system established by running four reference sera (A, B, C and D) with reference discs during each assay in parallel to the unknown samples. In the present study Class 1 or more was considered positive.

Direct skin tests

Prick tests were carried out on all individuals with the scabies mite extract and with thirteen commercially available allergen extracts, obtained from ALK, namely *Dermatophagoides pteronyssinus* (1:100), house dust (1:20), horse dander (1:100), dog dander (1:20), cat dander (1:20), cow dander (1:20), *Cladosporium herbarum* (1:20), birch pollen (1:100), mugwort pollen (1:100), timothy pollen (1:100), skimmed cow's milk (1:20), cod (1:20), and egg white (1:100). Intracutaneous injections were subsequently done with the same scabies mite extract and with the extract of *Dermatophagoides pteronyssinus*.

Both types of skin tests were performed using disposable hypodermic needles, size 20 (16 mm 5/10, Gillette Scimitar), and with an accepted technique (Pepys, 1972; Voorhorst & Vankreiken, 1973). The prick tests were performed on symmetrical anatomically corresponding sites on the upper back of the patients. Intracutaneous tests were performed on the lower arms with injections of approximately 0·02 ml solution.

The reactions were recorded after 20 min, 24 and 48 h. As positive (+ + +) reference, histamine HCl 1 mg/ml was used for prick tests and 0·1 mg/ml for intracutaneous tests. For skin testing 0·9% NaCl with 0·5% phenol was used as a negative (–) control.

The reactions were recorded as 3+ (+ + +) when the weals were of the same order of size as that of the histamine reference, with the size of the control site weal substracted. They were recorded as negative (–) when showing the same order of size as the corresponding negative control. Gradings 1+ 2+ 4+ etc. were used in relation to the histamine reference reaction, so that each additional (+) indicated an approximate doubling of the reaction (Aas & Belin 1974). The outlines of the weals were marked with a ballpoint pen and the marks were transferred to record sheets by means of a transparent tape. The sum of the largest diameter and the diameter perpendicular to it (D+d) in millimetres was used in weal measurements (Aas & Belin 1974). The result was recorded as positive when the reaction was 2+ or more.

Prausnitz-Küstner (P.K.) test

Two non-atopic control individuals who had never had scabies served as recipients in passive transfer experiments (P.K. recipients). Serum from five patients with scabies, two of them atopic individuals (both with RAST Class 4 to DP) and three patients with no history of atopy, but with RAST Class 2, 1, and 1 to DP, respectively were used in the P.K. test.

The injection sites of undamaged skin on the back of the recipients were marked with a ballpoint pen and 0·1 ml undiluted serum from each of the five patients was injected intracutaneously with controls as described under direct skin tests. The weals resulting from the injections were encircled with the ballpoint pen. No reactions to the serum injections could be observed during the next 24 h, after which 0·02 ml of the scabies mite extract was injected intracutaneously exactly in the middle of the encircled area. The reactions were recorded after 20 min. The outlines of erythema and weals were transferred to record sheets as described above.

Prick tests and intracutaneous injections of the scabies mite extract in unsensitized skin and of 0·9% NaCl in sensitized skin served as negative controls. In addition serum from each recipient served as an additional negative control. As positive reference (+ + +), histamine HCl 0·1 mg/ml for intracutaneous tests was used, whereas 0·9% NaCl with 0·5% phenol was used as a negative (–) control.

RESULTS

The prick tests to the scabies mite extract were negative in all cases. However, seven of the twelve individuals who had been treated for scabies had a positive intracutaneous reaction of the immediate type to the scabies mite extract (Table 1). All of the individuals demonstrating a positive reaction had had their scabies infestation 2–8 months before, whereas those with negative reactions had had their scabies 15–24 months previously. In no case could a delayed, Type IV, hypersensitivity reaction be observed. With only one exception the individuals with a positive intracutaneous reaction to the scabies mite extract also had positive intracutaneous reactions to DP, and five of these also had a positive prick test to DP (Table 1). On the contrary, the individuals with negative reaction to the scabies mite extract also had negative prick and intracutaneous tests to DP. Positive prick tests to one or more of the other common plant or animal allergens did not parallel these findings (Table 1). A

TABLE 1. Skin tests to a scabies mite extract and to extracts of *Dermatophagoides pteronyssinus* (DP) and other allergens* in a group of patients treated for scabies and in two control groups, one with no history of atopy and otherwise healthy, the other with an established skin sensitivity to DP. None of the controls had a history of scabies infection

Subjects	Intracutaneous test to SS	Prick test to DP	Intracutaneous test to DP	Prick test to one or more of other allergens	History of atopy
Patients					
1	++++	++	+++	+++	—
2	+++	—	+	++++	+
3	+++	+++	++++	++	+
4	++(+)	+	++(+)	—	—
5	++	++	++	—	—
6	++	++	+++	+++	+
7	++	+++	+++	+++	+
8	+	—	+	+++	—
9	+	—	+	—	—
10	(+)	—	(+)	—	—
11	—	—	—	—	—
12	—	—	—	—	—
Healthy controls 1–6	—	—	—	—	—
Controls with skin sensitivity to DP					
1	+++	+++	++++	—	+
2	++	++	+++	—	+
3	++	++	+++	+++	+

* See text.

history of atopy, consisting of allergic rhinitis, was found in just four of the patients, all of whom had positive skin tests to both *Sarcoptes scabiei* (SS) and DP, as well as to animal dander and pollen. Another four with no history of atopy had positive RASTs to DP, and two of these had positive skin tests only to SS and DP allergens. The serum concentrations of IgE were elevated in only two (> 1000 and 180 u/ml, respectively) of the twelve patients.

In the six healthy controls all intracutaneous tests with the scabies mite extract were negative (Table 1). On the other hand, all three controls with skin sensitivity to DP but no history of previous scabies had positive intracutaneous reactions to the scabies mite extract.

Positive reactions to the scabies mite extract could be demonstrated within 20 min in one P.K. test recipient with three of five sera injected and in the second recipient with both sera injected (Table 2). Prick tests and intracutaneous injections with the scabies mite extract and with 0·9% NaCl proved negative in unsensitized skin in both the P.K. test recipients. The normal serum controls were negative. All sera giving a positive P.K. test had RASTs to DP of Class 2–4, whereas in both sera with negative P.K. test RAST to DP was of Class 1.

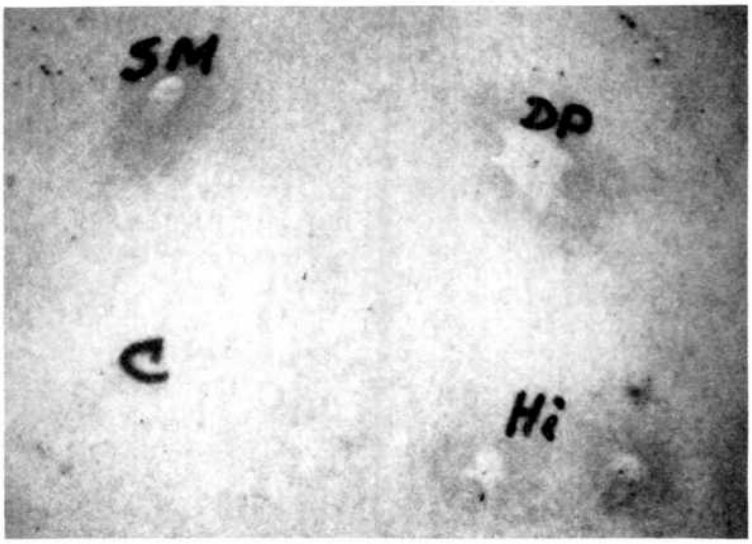

FIGURE 1. Positive intracutaneous test to the scabies mite extract. Hi = histamine 1:10000 (+++); SM = scabies mite extract 1:2500 (+++); DP 1:1000 (++++); C = control with sterile NaCl 0·9% with phenol 0·5% (–).

TABLE 2. Prausnitz-Küstner tests in two recipients with serum from patients with scabies and a scabies mite extract

Serum from:		Recipient No. 1	Recipient No. 2
Patient	1	+++	+++
	2	+++	+++
	3	++	ND
	4	(+)	ND
	5	(+)	ND
Recipient	1		—
	2	—	

ND = not done.

DISCUSSION

The positive intracutaneous reactions to scabies mite extract in more than half of our patients treated for scabies indicate that an immediate type immune reaction is initiated by this infestation. Since all the individuals with positive reactions had had their scabies infection less than a year previously, whereas those with negative reactions had had scabies more than a year before, a causal relationship is even more likely. The fact that the prick tests with the same extract were negative may be explained by insufficient allergenic activity of the allergen extract since only 5% of the total dry weight of the original material (scabies mites) is expected to be protein (S. Dale, personal communication), according to results from similar extraction procedures with house dust mites.

Intradermal injections of scabies mite extracts have previously given doubtful and contradictory results. Inoculation of crushed mites has resulted in pyogenic eruptions in normal skin (see for example Bourguignon, 1846; 1852; Gerlach, 1857), whereas no corresponding reactions at all were found by Sézary *et al.* (1931). Positive immediate type reactions have been found in allergic patients with scabies and in patients who previously had suffered from scabies (Heilesen, 1946). However, no reactions were seen following intradermal injection of scabies mite extracts in patients with scabies (Sézary *et al.*, 1931; Glaouberson *et al.*, 1940; Berna, 1942), but positive delayed hypersensitivity reactions have been reported by Kitchevatz & Pochtitch (1934), and Mellanby (1944). The proportion of positive delayed reactions increased with increasing duration of the disease.

It must be emphasized that in our investigations we had such small quantities of mite material available that it was not possible to undertake a purification of the extract. Thus, the presence of antigens other than the scabies mite antigen and also small molecular irritants might be possible. However, according to our control methods, as has been demonstrated earlier (Havnen, 1974; Frostad *et al.*, 1977), we believe that the reactions observed represent a specific immunological reaction. The specificity is confirmed by the results of the P.K. tests, which indicate the presence of reaginic antibodies to the scabies mite extract and/or DP in the sera of patients with scabies.

IgE antibodies may be localized solely to the mast cells in the skin, without surplus of IgE antibodies in the serum, which means that direct skin tests are positive whereas RASTs and P.K. tests are negative. This suggestion might explain the fact that the P.K. test was negative in two cases.

Other allergen specific antibody classes with reaginic qualities, besides IgE, may also play a role in the pathogenesis of scabies and explain the different findings in different patients with scabies.

Our previous demonstration of increased serum IgE levels in patients with scabies (Falk, 1980), as well as a very high proportion with circulating IgE antibodies to DP, indicates a cross allergenicity between SS and DP (Falk & Bolle, 1980). Our recent finding that a positive skin reaction to the scabies mite extract correlated well with a positive reaction to DP gives additional evidence for a cross allergenicity between SS and DP. In further support of this are our results of positive skin tests to SS in individuals with skin sensitivity to DP but with no history of previous scabies infestation.

In cases with both SS and DP antibodies it is possible that the reaginic sensitization may have been initiated by the DP or SS allergen to which the particular person was exposed first. To elucidate this, skin testing and RAST to both DP and SS allergen extracts should be carried out as early as possible in new cases of scabies.

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Streptococcus milleri causing treatable infection in perineal hidradenitis suppurativa

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SUMMARY

Streptococcus milleri was isolated from the active lesions of three patients with perineal hidradenitis suppurativa. In each patient, elimination of this organism by appropriate antibiotic therapy was accompanied by marked clinical improvement.

Hidradenitis suppurativa is a chronic relapsing suppurative condition of apocrine gland follicles. Apocrine glands usually open into pilosebaceous follicles, and occur normally in the skin of the axilla, buttocks, perineum, external genitalia, and breasts, and occasionally in other sites (Montagna, 1962). The pathogenesis of hidradenitis suppurativa is not well understood (Gordon, 1978, Roberts & Rook, 1979). The initial event is believed to be occlusion of the mouth of the apocrine gland follicle. The frequent presence of grouped comedones in affected areas suggests an underlying structural abnormality of the pilosebaceous unit. Inflammatory changes occur deep to the obstruction. In severe cases the gland is destroyed, and marked induration and scarring ensue with chronic sinus formation. The importance of bacterial infection is not clear. *Staphylococcus aureus* is sometimes isolated, and anaerobes have recently been implicated in axillary hidradenitis suppurativa (Leach *et al.*, 1979).

We report our preliminary findings implicating *Streptococcus milleri* in three patients with chronic severe hidradenitis suppurativa of the anogenital region, with an encouraging response to treatment.

CASE REPORTS

Case 1

Clinical details are shown in Table 1.

This 32-year-old man was referred for a final dermatological opinion before major surgery (Fig. 1). He had suffered from hidradenitis suppurativa of the buttocks and natal cleft for 16 years. For 18 months painful abscesses had made sitting virtually impossible, and he had been unable to work for a year. Profuse purulent discharge rendered him housebound, and he had expressed suicidal intentions. Extensive immunological investigation had failed to reveal any defect of polymorph function or of humoral or cellular immunity.

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TABLE 1. Bacterial findings, treatment and clinical progress in Case 1. Clinical activity was assessed on the amount of discharge, pain and tenderness.

Date	Antibiotics at time of investigation (total daily dose)	Clinical state at time of investigation	*Strep. milleri* in lesional discharge	*Strep. milleri* in stool or anal swab	*Strep. milleri* in other sites	Antibiotics after investigation (total daily dose)	Subsequent clinical course
23.10.78	Clindamycin (300 mg) Metronidazole (1·2 g)	Very active	3+ R Clindamycin R Erythromycin S Tetracyclines S Penicillin	Present R Clindamycin R Erythromycin		Nystatin (4,000,000 units, Weeks 1 and 2) Vancomycin (2 g, week 2) and neomycin (2 g, week 2) Gentamicin (240 mg, week 2) Benzylpenicillin (16 megaunits, Week 2)	Minimal improvement
4.11.78	Nystatin (4,000,000 units) Vancomycin (2 g) Neomycin (2 g) Gentamicin (240 mg) Benzylpenicillin (16 megaunits)	Very active	2+ R Clindamycin R Erythromycin S Tetracyclines S Penicillin	Absent		Doxycycline (200 mg, Day 1; 100 mg thereafter)	Excellent improvement
24.11.78	Doxycycline (100 mg)	Minimal activity	+ R Clindamycin R Erythromycin S Tetracyclines S Penicillin	Absent	Absent from mouth	Doxycyline (100 mg)	Improvement maintained
29.12.78	Doxycycline (100 mg)	Minimal activity	Absent			None	Improvement maintained
27.7.79	None	Minimal activity	3+ R Erythromycin R Clindamycin R Tetracyclines S Penicillin		Present in mouth R Tetracyclines S Clindamycin S Erythromycin S Penicillin	None	Improvement maintained

R = resistant to; S = sensitive to; + = light growth; 2+ = moderate growth; 3+ = heavy growth.

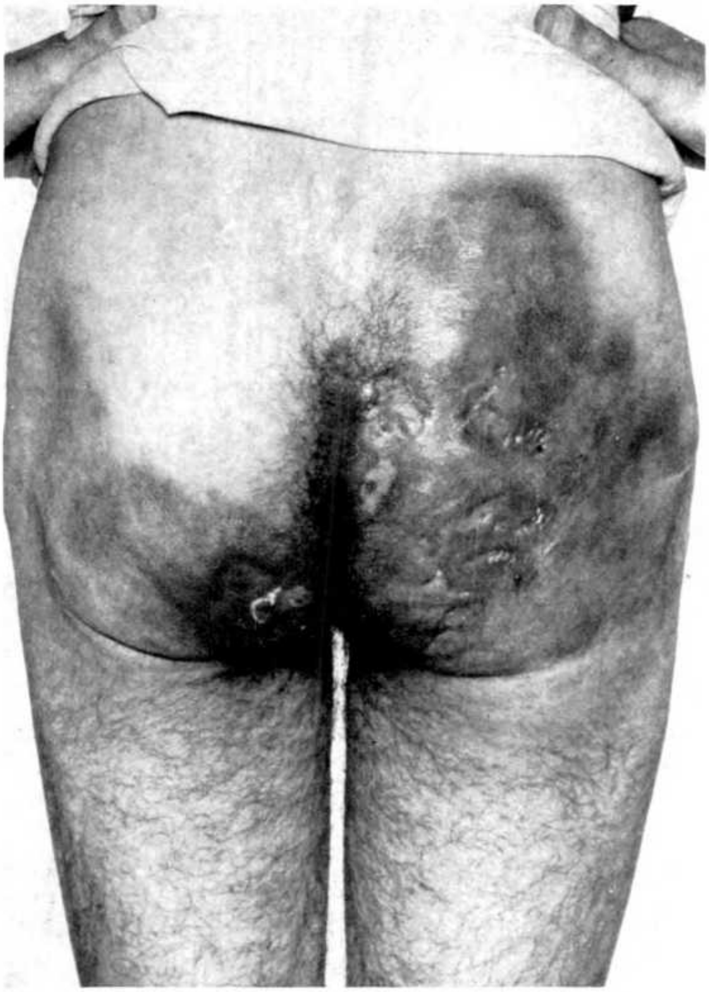

FIGURE 1. Buttocks of Case 1.

TABLE 2. Previous streptococcal isolates from active lesions of Case 1

Date	Streptococcal isolate
2.1.76	Haemolytic Group G
28.4.76	'Nonhaemolytic'
30.4.76	'Microaerophilic'
15.10.76	Haemolytic Group F — erythromycin resistant
7.10.77	'Microaerophilic'
11.2.78	β-Haemolytic not Group A, C, G — erythromycin resistant
23.10.78	Haemolytic Group F — erythromycin resistant (see Table 1)

TABLE 3. Bacterial findings, treatment and clinical progress in Case 2

Date	Antibiotics at time of investigation (total daily dose)	Clinical state at time of investigation	*Strep. milleri* in lesional discharge	*Strep. milleri* in stool or anal swab	*Strep. milleri* in other sites	Antibiotics after investigation (total daily dose)	Subsequent clinical course
5.2.79	None	Very active	2+ S Tetracyclines S Erythromycin S Clindamycin S Penicillin	Present		Nystatin (2,000,000 units, Days 1–7) Vancomycin (250 mg) and Neomycin (2 g) (Days 2–7) Doxycycline (200 mg, Day 8; 100 mg, Day 9 onwards)	Rapid improvement
1.5.79	Doxycycline (100 mg)	Minimal activity	Absent	Absent		None	Improvement maintained

TABLE 4. Bacterial findings, treatment and clinical progress in Case 3

Date	Antibiotics at time of investigation (total daily dose)	Clinical state at time of investigation	*Strep. milleri* in lesional discharge	*Strep. milleri* in stool for anal swab	*Strep. milleri* in other sites	Antibiotics after investigation (total daily dose)	Subsequent clinical course
14.2.79	Doxycycline (100 mg)	Active	Probably present (as 'microaerophilic *Streptococcus*) R Tetracyclines R Metronidazole S Clindamycin S Penicillin			Doxycycline (100 mg) Flucloxacillin (2 g)	Slight improvement
26.2.79	Doxycycline (100 mg) Flucloxacillin (2 g)	Moderate activity	+ R Tetracyclines S Clindamycin S Penicillin	Absent	Mouth: absent	Erythromycin (2 g)	Substantial improvement
11.6.79	Erythromycin (2 g)	Minimal activity	No pus obtained			None	Well for 2 weeks then moderate relapse
6.8.79	None	Moderate activity	Present	Present	Mouth: 2 strains present:both S Tetracyclines S Erythromycin S Penicillin	Erythromycin (2 g)	Moderate improvement

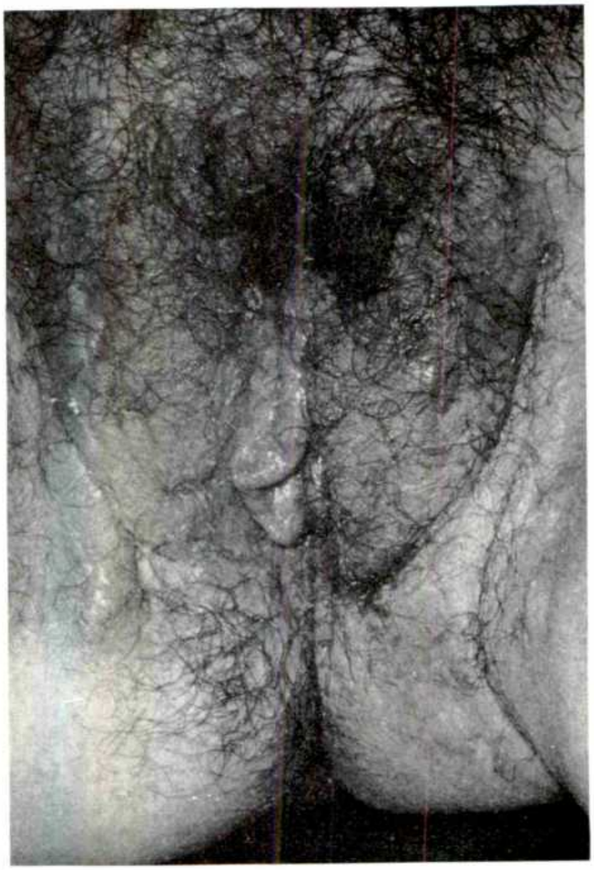

FIGURE 2. Vulva and perineum of Case 3.

The unusual severity of the disease prompted careful bacteriological investigation leading to the isolation of *Strep. milleri*. A review of previous bacterial culture results suggested that this streptococcus had previously been repeatedly isolated, but its importance not recognized or its identity not specifically confirmed (Table 2).

Case 2

Details shown in Table 3.

This 32-year-old man had a 12-year history of hidradenitis suppurativa of the perianal skin and the medial aspects of both buttocks. There was much malodorous discharge and considerable pain.

Case 3

Details shown in Table 4.

The initial manifestations of hidradenitis suppurativa in this 31-year-old woman were recurrent abscesses in and around the breast areolae beginning at the age of 18. Five years later the vulva, groins, perineum, perianal region, and axillae became involved. During the period of our investigation, significant disease activity affected the vulva and perineum only (Fig. 2).

None of these three patients gave a family history of hidradenitis suppurativa, and none reported a personal or a family history of acne of unusual type or severity.

DISCUSSION

Leach *et al.* (1979) reviewed their bacteriological findings in fifty-two patients with axillary abscess In twelve cases, all with axillary hidradenitis suppurativa, anaerobic bacteria, especially Gram-positive cocci, were isolated, but *Strep. milleri* was found in only one of these. Our experience in three consecutive cases of anogenital hidradenitis suppurativa is quite different in that *Strep. milleri* was isolated from active lesions in each patient. If this finding is confirmed, it would have important implications for treatment.

Strep. milleri is a homogeneous biochemically defined taxon of streptococci (Lütticken *et al.*, 1978). The organism may be β-, α-, or non-haemolytic, although use of these terms may disguise the fact that an organism has not been identified to species level. Its growth on primary inoculation is often dependent on or enhanced by incubation in 5% carbon dioxide. It is not an obligate anaerobe and is metronidazole resistant. It has been termed *Peptostreptococcus intermedius* in many American papers, which is unfortunate, as the genus *Peptostreptococcus* is usually considered to be strictly anaerobic and metronidazole sensitive. When grown on blood agar *Strep. milleri* often has a characteristic caramel odour. The organism commonly carries Lancefield Group F antigen, although 'minute' strains of streptococci carrying the Lancefield Group A, C or G antigens, or no detectable Lancefield Group antigen, are also included in the taxon. Rapid Lancefield Grouping techniques for Group F streptococci have only recently become commercially available. Only *Strep. milleri* bearing Group F antigen were sought in the three patients we describe.

Poole & Wilson (1979) detected *Strep. milleri* as a commensal in healthy subjects in the throat (31%), on extracted teeth (28%), in the vagina (18%), and in the faeces (15%). It is now one of the commonest isolates from abscesses of brain (de Louvois, 1978), lung (Bartlett & Finegold, 1972), pleura (Bartlett *et al.*, 1974), liver (Sabbaj, Sutter & Finegold, 1972; Bateman, Eykyn & Phillips, 1975) and other intra-abdominal sites (Poole & Wilson, 1976). In addition, it has been isolated in Meleney's synergistic bacterial gangrene (Meleney, 1931) and in our laboratory from patients with otitis media, paronychia, or pilonidal sinuses. There are no recent reports on its resistance to antibiotics. Our records based on susceptibility testing with discs of tetracycline (10 μg), erythromycin (5 μg), clindamycin (2 μg) and penicillin (1 unit) show the incidences of resistance in isolates from individual patients to be:

tetracyclines 87 of 240 = 36% resistant
erythromycin 4 of 160 = 2·5% resistant
clindamycin 1 of 92 = 1% resistant
penicillin 0 of 240 = 0% resistant.

However, as Case 1 illustrates, benzylpenicillin in high dosage, even when combined with an aminoglycoside, gentamicin, does not seem to be effective in eliminating the organism from these deep-seated dermal sinuses. The reason for this is not clear, but it is possible that the drug does not penetrate lesions well, either because of scarring or because of poor lipid solubility. In streptococci cross-resistance between erythromycin and clindamycin is the rule (Dixon & Lipinski, 1979), although resistance to these antibiotics is not common. Susceptibility testing of Gram-positive organisms to trimethoprim is often not reproducible between laboratories and may therefore not be a reliable guide to therapy.

In each of these three cases of perineal hidradenitis suppurativa, at a time of active disease with marked tenderness and purulent discharge, *Strep. milleri* was isolated as the predominant or only bacterium from lesional pus. Case 2 was on no antibiotic when investigated. In each of Cases 1 and 3 the *Strep. milleri* initially found was resistant to the antibiotics then being given, namely clindamycin (and, of course, metronidazole) in Case 1, and doxycycline in Case 3. Administration of an antibiotic to which the patient's organism was sensitive was followed by marked improvement in each case; in Case 1 doxycycline appears to have been the most effective drug, and Case 3 improved on erythromycin. It was noted, however, that lesions in Case 1 subsequently yielded *Strep. milleri*, a strain now resistant to tetracyclines, as well as to erythromycin and clindamycin, without clinical relapse.

It is interesting that the best results were in Cases 1 and 2 in whom *Strep. milleri* was eliminated from the stool following oral vancomycin (with neomycin to obtain bactericidal synergy and nystatin to prevent candidiasis). In Case 3, the organism was not found in the faeces initially and vancomycin was not given. This patient relapsed soon after stopping erythromycin and her stool was then found to contain *Strep. milleri*. It may be that treatment aimed at eliminating *Strep. milleri* from the gut should be given even if the initial stool culture is negative. A simple regimen seems desirable and the agent used should not select for a resistant flora. If subsequent work suggests that elimination of *Strep. milleri* from the gut is indeed necessary for effective therapy of hidradenitis suppurativa infected with that organism, vancomycin alone may be useful. Our addition of neomycin and nystatin may prove to be unnecessary. Vaginal carriage may require separate treatment.

FURTHER FINDINGS

Our investigations have now included a further ten patients with active anogenital hidradenitis suppurativa. In five of these ten cases, *Strep. milleri* was isolated from lesions as the predominant organism.

CONCLUSION

These preliminary observations suggest that infection with *Strep. milleri*, a frequent commensal in the gastrointestinal and female genital tracts, may cause readily treatable acute exacerbations in chronic hidradenitis suppurativa of the anogenital region. The simplest explanation is that the organism gains access to pre-existing sinuses in areas of scarring. We have not studied the possible role of *Strep. milleri* in the early stages of hidradenitis suppurativa.

Routine laboratory methods may fail to provide accurate identification of *Strep. milleri*, and collaboration between clinician and bacteriologist is advised. Sensitivity testing (to erythromycin and tetracyclines in particular) should permit effective treatment of these acute exacerbations.

Strep. milleri appears to be less important in axillary hidradenitis suppurativa (Leach *et al.*, 1979) and it seems likely that perineal lesions are more readily colonzied from the gut or the vagina. Treatment aimed at eliminating the organism from the flora appears logical, but we have insufficient evidence at present to confirm its value.

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An analysis of sebum excretion rate, bacterial population and the production rate of free fatty acids on human skin

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SUMMARY

Bacteria were sampled using a 'scrub' technique from the skin surface of the faces of forty-nine female subjects aged 18–21 years. The sebum excretion rate was determined by a gravimetric method and the level of free fatty acids by titration. The production rate of free fatty acids was calculated from the product of the concentration of free fatty acids in the sebum and the sebum excretion rate.

The date were analysed using Kendall's rank correlation method. Positive correlations existed between the number of *Micrococcaceae* and the skin propionibacteria ($P<0{\cdot}001$) and between both groups of organisms and the production rate of free fatty acids ($P<0{\cdot}001$). There was no significant correlation between the size of the bacterial population and the sebum excretion rate. The results support the view that free fatty acids are produced as a result of bacterial action, that the *Micrococcaceae* and skin propionibacteria do not compete to the detriment of their respective populations, and that the size of the bacterial population is not dependent upon the sebum excretion rate.

The resident skin microflora is a stable ecological system in which bacterial strains may persist in relatively constant numbers for considerable periods of time (Evans, 1975). A number of separate studies have examined factors which may affect the size or composition of the bacterial population e.g. age and sex (Leyden *et al.*, 1975), the area of skin sampled (McGinley, Webster & Leyden, 1978) and antibiotic therapy (Marples & Kligman, 1971).

Marples *et al.* (1970) and Marples, Downing & Kligman (1971) reported that the presence of free fatty acids on the skin was due to the action of propionibacterial lipase on sebum triglycerides and not due to lipases produced by members of the *Micrococcaceae*.

It is possible that sebum constituents or their breakdown products are of nutritional importance to the microorganisms (Weeks, *et al.*, 1977; Ferguson & Cummins, 1978). Therefore, the size of the bacterial population may be directly related to the sebum excretion rate. The objective of this work was to determine in a single study whether correlations exist between the size of the major con-

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stituents of the bacterial population, the sebum excretion rate and the rate of production of free fatty acids.

MATERIALS AND METHODS

Subjects

Forty-nine female subjects aged 18–21 years with mild to moderate acne were involved in this study. They received no oral or topical therapy for at least 1 month prior to the investigation.

Bacteriological methods

Bacteria were sampled from the face using the 'scrub' technique (Williamson & Kligman, 1965). Colony forming units of skin propionibacteria and members of the family Micrococcaceae were determined as previously described (Cove, Cunliffe & Holland, 1980). Bacterial numbers were expressed as colony forming units cm^{-2} skin.

Sebum excretion rate

The sebum excretion rate was determined on the forehead by the gravimetric method, employing absorbent papers as described by Strauss & Pochi (1961) and modified by Cunliffe & Shuster (1969). The sebum excretion rate was expressed as ng sebum cm^{-2} skin min^{-1}.

The production rate of free fatty acids

Sebum extracted from the absorbent papers was reconstituted in 5 ml heptane and the free fatty acids extracted by the method of Dole & Meinertz (1960). The fatty acid concentration was then determined by titration against alkali (N/200 tetra-n-butyl ammonium hydroxide dissolved in redistilled methanol) using an autoburette (Radiometer, Copenhagen). The production rate of free fatty acids was calculated from the product of the concentration of free fatty acids in the sebum and the sebum excretion rate; this was expressed as ng free fatty acids cm^{-2} skin min^{-1}.

RESULTS

The geometric mean bacterial population, the mean sebum excretion rate and the production rate of fatty acids are given in Table 1. The data were further analysed for correlations between the bacterial population, sebum excretion rate and the production of free fatty acids using Kendall's rank correlation (Kendall, 1970). This method was employed since it does not require the data to fall into a normal distribution and a log transformation of the data does not affect the result. The τ

TABLE 1. The bacterial population, sebum excretion rate and production rate of free fatty acids on the faces of forty-nine female subjects

	Mean data
Propionibacteria	$4{\cdot}2 \pm 0{\cdot}21$ $\log_{10}$ C.F.U. cm^{-2}
Micrococcaceae	$3{\cdot}7 \pm 0{\cdot}15$ $\log_{10}$ C.F.U. cm^{-2}
Sebum excretion rate	1004 ± 61 ng cm^{-2} min^{-1}
Production rate of free fatty acids	175 ± 22 ng cm^{-2} min^{-1}

TABLE 2. Analysis of the bacterial population, sebum excretion rate, and production rate of free fatty acids of forty-nine female subjects using Kendall's rank correlation

	τ	P Value	
Micrococcaceae compared to skin propionibacteria	0·41	<0·001	(S)
Micrococcaceae compared to FFA	0·31	0·001	(S)
Micrococcaceae compared to SER	0·07	0·317	(NS)
Skin propionibacteria compared to FFA	0·43	<0·001	(S)
Skin propionibacteria compared to SER	0·08	>0·50	(NS)

FFA: Production rate of free faty acids (ng cm^{-2} min^{-1}).
SER: sebum excretion rate (ng cm^{-2} min^{-1}).

values and probabilities are given in Table 2. Positive correlations existed between the number of *Micrococcaceae* and the skin propionibacteria, the number of *Micrococcaceae* and the production rate of free fatty acids, and the number of skin propionibacteria and the production rate of free fatty acids. Clearly these correlations were not perfect and for unknown reasons in some cases high numbers of one bacterial type occurred at the same site with low numbers of the other type. No correlation was found between the sebum excretion rate and bacterial numbers.

DISCUSSION

It was important to consider the rate of production of free fatty acids and not the concentration of free fatty acids in the sebum since this represents more accurately the extent of possible bacterial activity on sebum triglycerides. An individual with a high sebum excretion rate may possess a low percentage of free fatty acids in the sebum and yet produce fatty acids at a higher rate than an individual with a low sebum excretion rate and a high percentage of fatty acids in the sebum. Significant correlations were shown between the number of *Micrococcaceae* and free fatty acid production and the number of propionibacteria and free fatty acid production. Previous workers (Marples, *et al.*, 1970, 1971) have shown that the concentration of free fatty acids in the sebum was related to the bacterial number and especially the propionibacteria. This investigation supports the view that the skin bacteria are responsible for the free fatty acid production. However, since there is a positive correlation between the population of *Micrococcaceae* and propionibacteria, it is not possible to determine whether one or both types of bacteria are responsible for the free fatty acid production.

Since there is a positive correlation between the numbers of *Micrococcaceae* and propionibacteria, it does suggest that one group of organisms do not grow at the expense of the other. Therefore, it is possible that the *Micrococcaceae* and the skin propionibacteria occupy different ecological niches and do not compete with each other for either space or nutrients. Other explanations can be considered. Firstly, *Micrococcaceae* and skin propionibacteria could compete, but overriding factors could predispose to a large or small total bacterial population. Such factors could be the availability of nutrients or the size of the pilosebaceous ducts. Secondly, one of the types of bacteria could have a beneficial effect on the other, i.e. commensalism. Thirdly, both types could be stimulatory to each other, i.e. protocooperation (Alexander, 1971). To determine which explantion is correct further experimentation is required.

No correlation was found between the sebum excretion rate and the size of the bacterial population. Therefore it was apparent that a high sebum excretion rate was not the factor predisposing to a large bacterial population and other factors must be limiting in the environment. This limitation may be due to either low availability of essential or stimulating substances in the environment or to a higher level of inhibiting substances in the environment. However, it is possible that the 'turnover' rate of bacteria and hence the bacterial growth rate is greater on skin with a high sebum excretion rate. As yet no satisfactory method has been devised for the determination of the growth rate of bacteria on the skin, but such a determination will lead to a better understanding of the ecology of skin bacteria.

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The effects on rat skin of prolonged exposure to water

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SUMMARY

By means of small waterproof chambers held on the backs of rats with adhesive, areas of skin were exposed continuously to sterile distilled water for 1-, 2- and 3-day periods. All test areas showed discernible changes and many exhibited vesicles, bullae or abscesses in the epidermis. Dermal changes ranged from mild inflammatory cell infiltration to severe oedema and haemorrhagic inflammation.

The effects of water and other substances on the skin have been studied in a number of ways (Blank & Dawes, 1958; Fritsch & Stoughton, 1963; Stillman, Hinson & Maibach, 1971; Fisher & Maibach, 1972; Bartek, LaBudde & Maibach, 1972; Weigand & Gaylor, 1973; Willis, 1973; Aly *et al.*, 1978; Kerbs & Allen, 1978). Recently, a new *in vivo* technique for studying the effects of fluids on skin was developed (Swan, 1975) and modified (Swan, 1976). In the present investigation, the latter technique was used to study the effects of water on the skin of rats.

MATERIALS AND METHODS

Young adult male albino rats were anaesthetized with intraperitoneal injections of 30 mg/kg Nembutal*, augmented with inspired ether if necessary. The hair on part of the back of each rat was removed with electric clippers and the upper incisors were shortened by 1½ mm and the lower incisors by 4 mm using a diamond disc in a dental handpiece. Two black polypropylene chambers were attached to the back of each rat as described previously (Swan, 1976). Sterile distilled water was introduced into the right side chambers which were sealed with stoppers. All left side chambers (controls) were left empty, some were sealed with stoppers, others left open to the air. The animals were caged in groups of six and fed powdered rat cubes. The water in the test chambers was emptied daily and the chambers immediately refilled with fresh water. The animals were killed at the end of 1-, 2- or 3-day test periods, water having been in the right chambers continuously.

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* Pentobarbitone sodium (Abbott).

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Nine rats (45 days old) were used for a 1-day test period, nine rats (44 days old) for a 2-day test period and eighteen rats (between 43 and 61 days old) for a 3-day test period. The larger number was used for the 3-day test in the event of some chambers being lost during this period. No chambers were lost during the 1- and 2-day periods but five were lost or leaked during the 3-day period, leaving thirteen in the experiment.

Skin specimens were harvested from the two test areas beneath the chambers on each animal, a third specimen was taken from the clipped skin between the chambers and a fourth from unclipped skin nearby. The specimens were prepared for paraffin sections and stained with haematoxylin and eosin.

RESULTS

Normal rat skin that has not been clipped (Fig. 1) has a thin layer of epithelium characterized by the following features. The basal cells range from flattened to low columnar but are mainly cuboidal. The spinous layer is usually only one or two cells in thickness and above this is a clearly defined granular layer some two to four cells thick. The abundant keratin on the surface is of two types: above the granular layer is a narrow, slightly eosinophilic stratum corneum conjunctum (S.C.C.) in which the squames are tightly packed and above this is a wide, basophilic stratum corneum disjunctum (S.C.D.) which has an open 'chicken-wire' or flakey appearance.

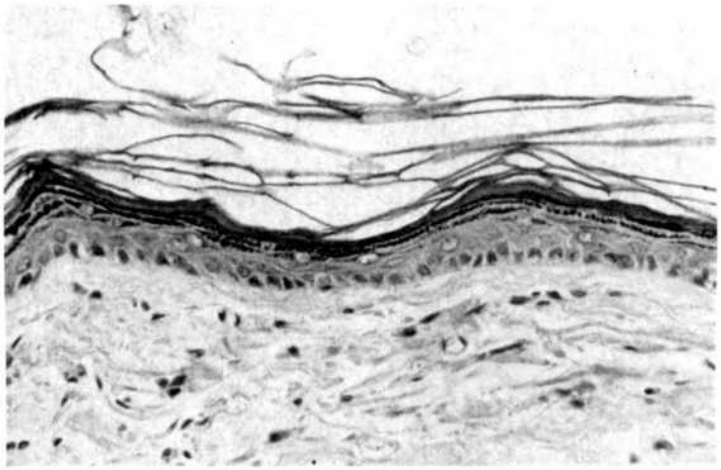
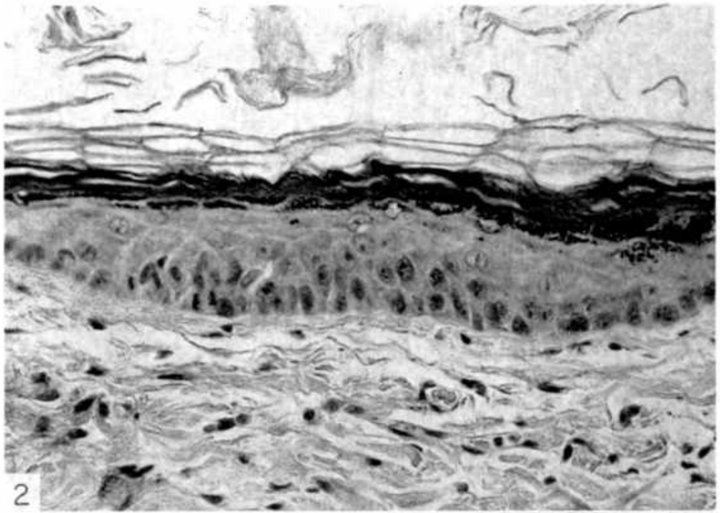


FIGURE 1. Normal unclipped rat skin (× 160).

No visual differences could be detected between sections taken from unclipped or clipped skin or skin taken from beneath empty chambers left open to the air. In specimens from beneath empty chambers sealed with screws the S.C.C. appeared slightly thickened.

Water exposed skin

All specimens in the 1-, 2- and 3-day tests showed some changes in the epidermis and some degree of inflammatory cell infiltrate in the dermis. In all water-exposed skin the S.C.D. was much thinner and the S.C.C. was thicker than normal. Changes in the cellular part of the epithelium (non-cornified epidermis) ranged from an increase in thickness alone, to severe destruction with vesiculation and bulla and abscess formation.



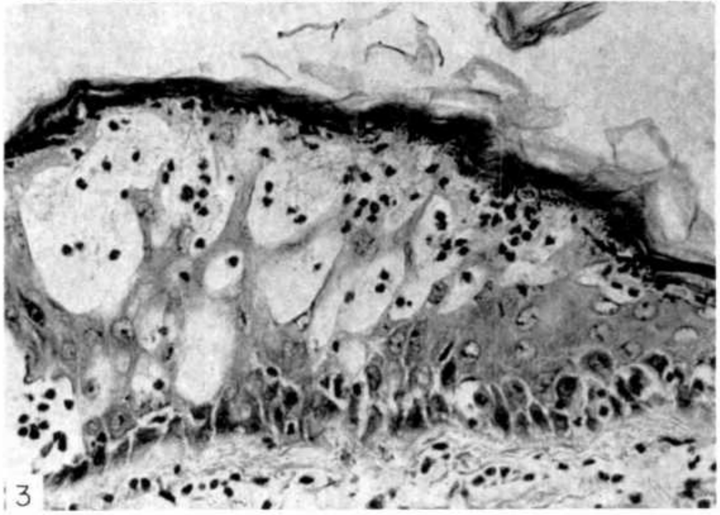


FIGURE 2. Epidermal thickening after 1 day's exposure to water (× 160).

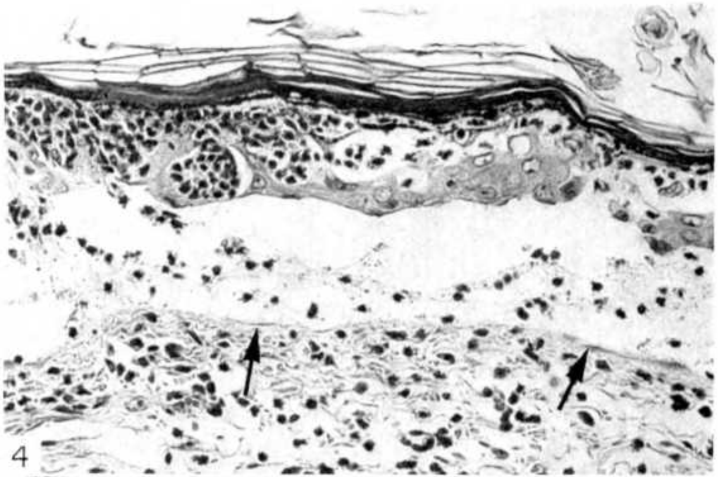
FIGURE 3. Vesicles and abscesses distributed throughout the full thickness of the epidermis resulting from 1 day's exposure to water × 160).

The terms vesicles, bullae and abscesses are used here to describe lesions observed in the epithelium because some were very small and contained no inflammatory cells; some were deemed large enough to be called bullae and some (small or large) contained sufficient polymorphs to warrant the term abscess. The grosser changes always occurred in the central part of the test area and the periphery generally showed milder changes.

1-Day test period. In three of the nine rats the whole of the water-treated areas showed intact epithelium in which the cellular part had thickened by a factor of approximately two as estimated by means of an eyepiece graticule in the microscope (Fig. 2). There was no apparent epithelial destruction in these specimens.

In the other six rats epithelial damage had occurred in the central one-sixth to one-half of the specimens in the form of vesiculation and abscess formation. The periphery of these test areas exhibited only the changes shown in Fig. 2. In five of these six animals bullae or abscesses involved the whole space between the basal layer and the keratin (Fig. 3) or there was a combination of supra-basal and subcorneal bullae or abscesses (Fig. 4). The periphery of most areas of severe destruction revealed superficial abscesses or bullae involving only granular and superficial spinous layers (Fig. 5). The test area in the remaining rat showed a number of very small abscesses located only in the granular and superficial spinous layers.

Measurements of the cellular part of the water-exposed epithelium, where no vesiculation or abscess formation had occurred, showed an average increase in thickness 2·2 times normal (range 1·9–2·4) for the nine rats. Where gross damage had occurred, with bulla and abscess formation, the non-cornified epithelium had increased up to 30 times normal thickness.



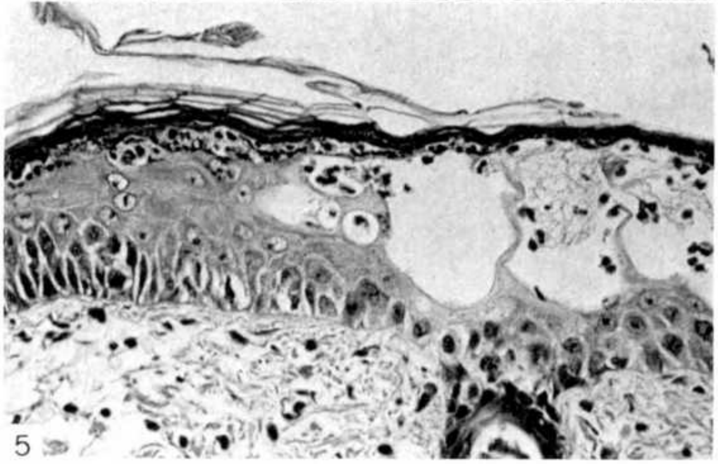


FIGURE 4. Numerous micro-abscesses beneath the keratin and a large supra-basal bulla caused by 1 day's exposure to water. Arrows indicate the basal layer. There is a moderate inflammatory cell infiltrate in the corium (× 160).

FIGURE 5. Sub-corneal micro-abscesses (left) at the periphery of an area of severe destruction (right) (× 160).

In all specimens but two, the corium showed a mild, mixed inflammatory cell infiltrate comprising mainly lymphocytes. In the other two the infiltrate was moderate and there was obvious oedema.

2-*Day test period*. Epithelial changes produced by water exposure for 2 days were essentially similar in incidence and extent to those seen in the one day specimens, except that the cellular part (away from bullae and abscesses) was estimated to have increased in thickness by a factor of three (range 2·6–3·8). Four of the nine rats showed thickening of the non-cornified epithelium and S.C.C. as the only changes and five had some intra-epithelial bulla or abscess formation.

The white cell infiltrate in the dermis was comparable with that in the one-day specimens but haemorrhagic inflammation was present also in some specimens. This extravasation of red cells was seen in four of the nine rats and ranged in intensity from a few small patches just beneath bullae to heavy concentrations of erythrocytes extending down to the muscle layer (Fig. 6).

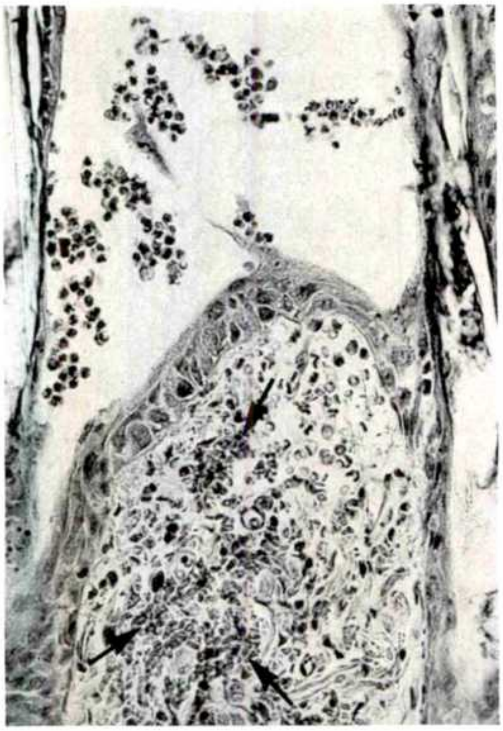


FIGURE 6. Two-day's exposure. Patches of red blood cells (arrowed) beneath an intra-epithelial bulla lying between two hair follicles (× 160).

Haemorrhagic inflammation was found only in specimens with intraepithelial bulla/abscess formation and two of the specimens had red blood cells in the bullae as well as in the dermis. Five specimens had dermal oedema—in four instances beneath epithelial bullae and in one, beneath thickened epithelium.

3-*Day test period*. After 3 days exposure to water, changes in the epidermis were similar in nature to those seen after 1 and 2 days but the incidence and extent of destruction were greater. In areas not affected by bulla or abscess formation the cellular epithelium was thicker than in the 1- and 2-day specimens and was estimated to show a 3·8 fold increase over normal thickness (range 2·7–5·8).

One specimen had no bulla or abscess formation and showed only thickening of the cellular epithelium and the S.C.C. The other twelve water-exposed specimens had bullae or abscesses in the subcorneal or suprabasal positions or both. In one rat these lesions occurred over the whole water-exposed area, while in the others they involved the central one-sixth to two-thirds. There was considerable variation in the degree of damage. Eight specimens had only subcorneal bullae and four showed extensive destruction of epithelial cells, including two with disruption of the basal layer where the epithelial bullae merged with severe dermal oedema (Fig. 7).

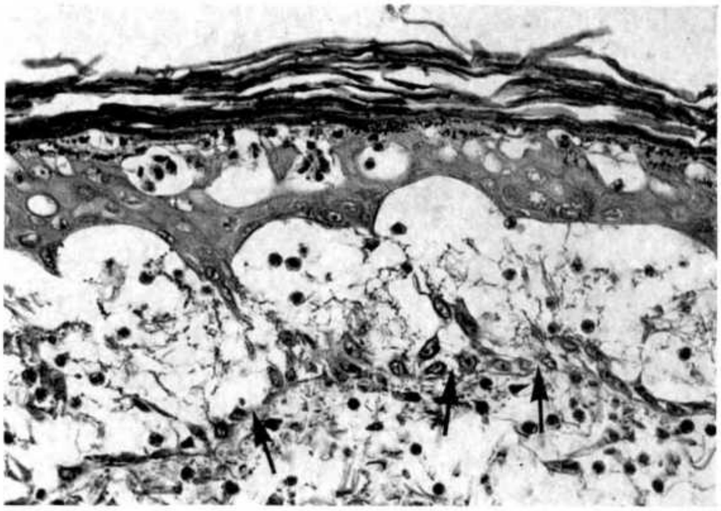


FIGURE 7. Three-day's exposure. Epithelial vesiculation and dermal oedema. Arrows indicate disrupted basal layer (× 160).

Inflammatory changes in the dermis were generally more severe and had penetrated deeper than in the 1- and 2-day test areas. Sub-epithelial oedema was found more frequently (nine of thirteen specimens) and was more extensive than in the 1- and 2-day samples.

Haemorrhagic inflammation was seen in six of the thirteen test areas and ranged from scattered patches of red blood cells to extensive areas of interstitial haemorrhage.

DISCUSSION

All test areas showed some change due to continuous contact with water (see Table 1). Even 1 day's exposure produced severe damage in some specimens and in general the degree of epithelial disruption was not greatly increased by 2 or 3 days' exposure. However, the effect on the sub-epithelial tissues was intensified by longer periods of contact with water.

From this study it was not possible to pinpoint with certainty the most susceptible part of the epithelium. Some specimens showed abscess formation only in the layer immediately beneath the keratin, some had abscesses and vesicles throughout the entire thickness of the non-cornified epithelium and two showed bullae and abscesses only in the suprabasal position. Nevertheless twelve of the total thirty-one rats had subcorneal abscesses only, and the periphery of most areas of severe damage showed vesiculation limited to the same region. These observations suggest that this could

TABLE 1. Effects on various test areas of rat skin due to continuous contact with water over 1-, 2- and 3-day periods

Rat No.	Exposure to water (days)	Cellular epithelium thickened	Subcorneal bullae/ abscesses	Intra-epithelial bullae/ abscesses	Supra-basal bullae/ abscesses	Dermal inflamm-atory cells	Dermal oedema	R.B.C. in dermis	R.B.C. in bullae
1	1	+				+			
2	1	+				+			
3	1	+	+	+	+	+			
4	1	+				+			
5	1	+	+		+	+	+		
6	1	+	+		+	+			
7	1	+	+			+			
8	1	+	+	+	+	+			
9	1	+	+	+	+	+	+		
10	2	+	+			+	+	+	+
11	2	+				+			
12	2	+				+	+		
13	2	+	+	+		+	+	+	
14	2	+	+	+	+	+	+	+	
15	2	+	+			+	+	+	+
16	2	+	+			+			
17	2	+				+			
18	2	+				+			
19	3	+	+			+	+	+	
20	3	+	+			+	+		
21	3	+				+	+		
22	3	+	+			+			
23	3	+	+			+	+	+	+
24	3	+	+			+		+	
25	3	+	+	+		+	+	+	+
26	3	+	+			+		+	
27	3	+	+			+	+		
28	3	+			+	+	+		
29	3	+			+	+	+	+	+
30	3	+	+	+	+	+	+		
31	3	+	+			+			

be the prime site of initial epithelial breakdown. The stratum corneum conjunctum and the basal layer of epithelial cells were apparently the structures most resistant to damage.

Some specimens in each of the 1-, 2- and 3-day test periods withstood water immersion very well and showed only thickening of the non-cornified epidermis and the S.C.C. together with dermal inflammation. That is, they had no apparent tissue destruction. A number of explanations could be advanced for the wide variation in response to water exposure but it is likely that the individual permeability of the various test areas played a major role. This in turn may have been due to several factors including the lipid-protein structure of the epithelium and the initial thickness of the S.C.C. which is thought by some to be the major barrier to water absorption through skin (Marzulli, 1963). There are others, however, who regard the entire stratum corneum as being uniformly impermeable, but much of this work was done on human skin which is distinctly different from rat skin (Scheuplein, 1976).

Further it may be postulated that thickening of the S.C.C. and the non-cornified epithelium are defensive mechanisms against water penetration and thus the individual ability of each animal to react in this way would be a factor in limiting water-caused damage. Thickening of the S.C.C. was always accompanied by thinning of the S.C.D. and this combined effect may have resulted from a number of factors. There may have been rapid shedding of S.C.D. and thickening of S.C.C. by hydration or by accretion from the granular layer. Alternatively, there may have been a reduction of the rate of transformation of S.C.C. to S.C.D. Less likely, but conceivably, there may have been 'rehydration' of the deep S.C.D. causing condensation and reattachment to S.C.C. A number of such mechanisms may of course have acted simultaneously.

The occurrence of fluid filled vesicles and bullae containing few inflammatory cells and none at all in some specimens and the finding of others heavily charged with polymorphonuclear leukocytes suggest that bacterial infection played little or no part in the former whilst it may have played a role in the latter. Thus it was inferred that vesiculation without abscess formation had been initiated by osmotic effects caused by water penetration.

The speed with which polymorphs could appear in the epithelium was notable in that six of the 1-day specimens had abscesses in the epithelium and one of these had abscesses only in the subcorneal layer. This was at least suggestive of early infection in those areas.

Infiltration of mixed inflammatory cells into the dermis was seen in all specimens, but in the majority the infiltrate was rated as only mild or moderate and it comprised mainly lymphocytes. The few instances of heavy inflammatory cell infiltrate were all associated with dermal oedema and haemorrhagic inflammation.

Sub-epithelial oedema was observed in two of the nine 1-day specimens (22%), five of the nine 2-day specimens (55%) and in nine of the thirteen 3-day ones (69%). Severity and extent of the oedema also increased with longer exposure to water.

Haemorrhagic inflammation was not seen in any 1-day specimens but was observed in four of the nine 2-day specimens (44%) and in seven of the thirteen 3-day specimens (54%). Red blood cells were seen also in epithelial bullae in two of the 2-day specimens and in three of the 3-day specimens, all of which had haemorrhagic inflammation. For extravasation of red blood cells to occur, vasculitis must first have been present. It is reasonable to infer that the presence of red blood cells in the dermis (whilst a non-specific sign) indicates rather severe inflammation causing damage to the walls of small blood vessels of the area. All observations on the dermis suggested that inflammatory changes increased with longer periods of exposure to water.

It was of note that severe changes could occur in the dermis in the absence of destruction of the epithelium, and also that deep parts of the epithelium could be destroyed without apparent damage in the superficial layers. For example, two 3-day specimens had severe suprabasal abscess/bulla formation without any overlying destruction in the epithelium. Also, several 3-day specimens had severe dermal oedema and haemorrhagic inflammation with relatively minor or superficial epithelial damage. Possibly this could be explained by water penetration along dermal appendages.

Far more investigation will be needed to understand the marked increase in thickness of the vital cellular part of the epithelium in response to water exposure. Its thickness increased by factors of approximately two, three and four for the 1-, 2- and 3-day specimens respectively. The simplest explanation is that this was a hydration effect with imbibition of water and enlargement of the individual cells and perhaps of the intercellular spaces. However, this was not supported histologically, because the number of layers of epithelial cells had also increased in the water-exposed skin. Thus, one is left to contemplate a rapid increase in cell production in response to water exposure or a reduction in transformation of superficial epithelial cells to keratin or a combination of the two.

Willis (1973) was able to expose areas of skin of human volunteers continuously to water for periods of 72–144 h. It is, however, somewhat difficult to relate the results of the present investigation with those of Willis because of the different types of skin used and the different points of emphasis in the two studies. Nevertheless, where comparisons can be made the two sets of results are not in disagreement. Willis reported a thickened horny layer, acanthosis, moderate to marked oedema of the upper dermis and mild to moderate mononuclear cell infiltrate in the dermis. He discounted bacterial growth, change in pH and anhidrosis as causative factors and suggested water itself as the most important irritating agent with a possible contribution from some potentially toxic natural surface substance such as urea.

In conclusion, the following points are made.

1-, 2- and 3-day exposure of living rat skin to sterile distilled water produced discernible changes in every case.

There was always thickening of the non-cornified part of the epithelium, together with an increase in the stratum corneum conjunctum and a decrease in the stratum corneum disjunctum.

The dermis always showed some degree of inflammatory cell infiltrate.

Some epithelial destruction occurred in most test areas (twenty-three of thirty-one, i.e. 74%) in the form of minor or major vesiculation or small or large abscess formation.

In some 1- and 2-day specimens epithelial damage was as severe as in the 3-day specimens but the proportion of 3-day specimens with epithelial damage was higher.

The degree and extent of inflammatory response in the dermis was, in general, proportional to the duration of water exposure. Most one-day specimens showed only mild inflammatory cell infiltrate but several three-day specimens exhibited severe, extensive oedema and haemorrhagic inflammation.

In some areas of epithelial disruption (vesiculation) there was no evidence of infection. In others, intra-epithelial abscesses were a feature.

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In vitro basal lamina formation may require non-epidermal cell living substrate*

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SUMMARY

In an adult human epidermal cell culture system in which three different types of dermo-epidermal junctions could be observed, the *in vitro* synthesis of basement membrane and type IV collagen was studied with electron microscopy and indirect immunofluorescence via an antiserum to type IV collagen. Essentially, keratinocytes juxtaposed to non-living substrate did not produce either type IV collagen or an ultrastructural basement membrane, whereas both products were found at dermo-epidermal junctions composed of living keratinocytes juxtaposed to living dermis. This suggests that the microenvironment of the keratinocyte may influence the synthesis of junctional components.

Within the four component dermo-epidermal junction (DEJ) the electron dense basal lamina (also called lamina densa and basement membrane) lies in between the electron lucent lamina lucida above, and the sub-basilar fibrillar area below (Briggaman & Wheeler, 1975). This structure, 200–500 Å thick and thought to be composed of a fine fibrillar material embedded within an electron dense matrix (Hodge & Freeman, 1978), is known to be rich in Type IV collagen (Yaoita, Foidart & Katz, 1978). Therefore, in addition to electron microscopic visualization, basal lamina can now be revealed with indirect immunofluorescence (IIF) techniques by reacting the structure against anti-Type IV collagen antibody. Previous *in vitro* studies have shown that this structure is synthesized by epidermal cells (Briggaman, Dalldorf & Wheeler, 1978; Marks, Abell & Nishikawa, 1975; Hintner *et al.*, 1979). Human skin explants, cultured by the Aaron Freeman culture technique, are known to produce a thick, keratinizing, stratified epidermal cell outgrowth that is juxtaposed to non-viable pig dermis (Freeman *et al.*, 1976) (Figs 1 and 2a). The epidermal outgrowth is capable of synthesizing bullous pemphigoid antigens (Woodley *et al.*, 1979) (Fig. 2b), and pemphigus antigens (unpublished observation). Using this system, we studied the possibility that the outgrowth epidermal cells would produce basal lamina and Type IV collagen. Therefore, we examined the epidermal cell outgrowth pig dermal

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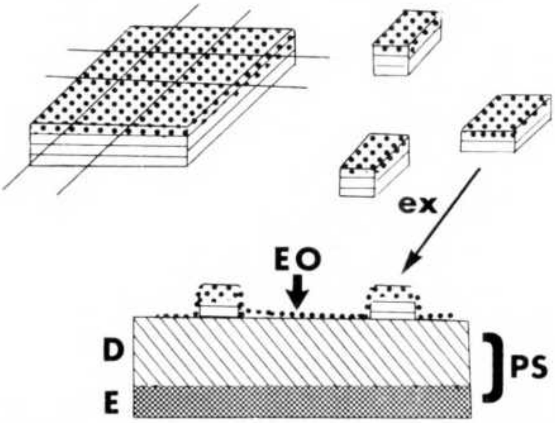

FIGURE 1. A schematic representation of the Freeman technique. Keratomed human skin is cut into explants (ex) and placed on the dermal (D) side of non-viable, inverted pig skin (PS). Epidermal cells from the explant migrate out onto the pig dermis forming an epidermal outgrowth (EO) juxtaposed to the dead pig dermis. E = pig skin epidermis.

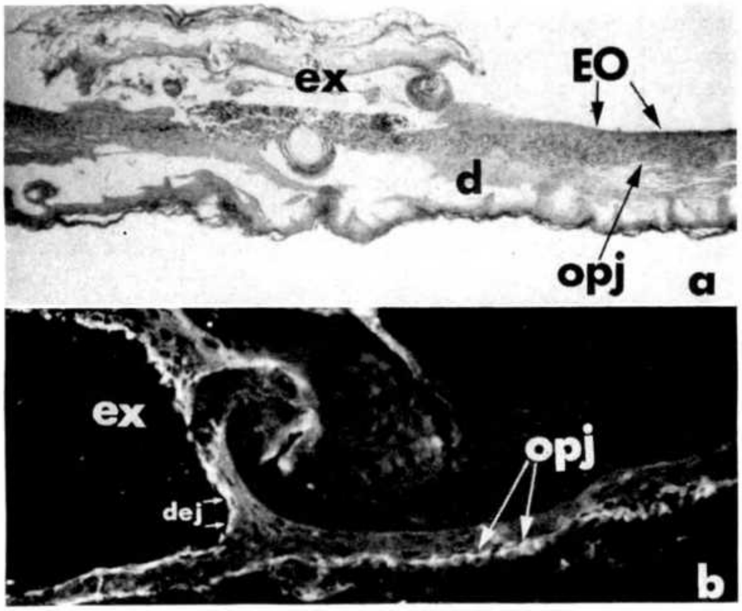

FIGURE 2.(a) A haematoxylin and eosin section of an explant-outgrowth unit after 12 days in culture (× 100), showing a deranged explant (ex) and an epidermal outgrowth (EO) juxtaposed to partially digested pig dermis (d). opj = outgrowth pig dermal junction. (b) An explant outgrowth unit from a previous study taken from culture on Day 20 and stained by IIF techniques with bullous pemphigoid (BP) anti-serum (× 250). BP antigen is expressed at the dermo–epibolic junction (dej) of the explant and the outgrowth pig dermal junction (opj). See Woodley *et al.* (1979) for further details of this study.

junction in this system with both the electron microscope and indirect immunofluorescence techniques against anti-Type IV collagen antibody.

MATERIALS AND METHODS

Cultures

All cultures were made in Eagle's minimal essential medium with 20 mM of Hepes Buffer and supplemented with 0·1 mM of non-essential amino acids, 1 mM of sodium pyruvate, 2 mM glutamine, 100 μg/ml streptomycin, 100 units/ml penicillin, and 10% fetal bovine calf sera.

Normal adult human skin was used in all studies. Skin was removed by keratome (0·3 mm depth) in thin sheets, cut into 2 mm × 2 mm squares, and placed onto pieces of inverted, non-viable pig skin (gamma irradiated with 3·5 Mrad and commercially available through The Burn Treatment Skin Bank, Phoenix, Arizona and Armour Laboratories, Paris, France). The specimens were incubated at 37°C and media changed twice a week.

Electron microscope

On Days 3, 5, 8, 16, 18, 21, 24, 30 and 37, explants and their epidermal outgrowths were taken from culture for examination with the electron microscope (EM). Specimens for EM were placed in Karnovsky fixative (2·5% glutaraldehyde and 22% paraformaldehyde in 0·33 M phosphate buffer). After postfixation in 1·33% osmium and 2% uranyl acetate for 2 h each, the specimens were desiccated with serial alcohol solutions, embedded in Epon and further processed in the usual manner (Karnovsky, 1965).

Indirect immunofluorescence

Specimens (explants and their epidermal outgrowth) were taken from culture on Days 10, 17 and 22, quick frozen in liquid nitrogen for 2 h and then stored at −20°C. Approximately 1 month later these specimens were sectioned with a cryostat microtome set at 4 μm. A methylene blue stained slide was made from each day to confirm a morphologically intact explant and outgrowth unit. All of the other sections were kept within the cryostat microtome machine at minus 30°C. The same day, they were packed (without thawing) in dry ice and taken by one of the authors to the Max-Planck Institute (this was graciously arranged by Dr Raul Fleischmajer and Dr Rupert Timpl) where the slides were brought to room temperature and allowed to dry for 10 min. The cryostat sections were then incubated for 30 min at room temperature with purified rabbit anti-mouse Type IV collagen antibody, protein concentration 30 μg/ml, in a moisture chamber. The slides were rinsed twice and washed for 2 min with PBS. They were subsequently incubated for 30 min with commercially prepared (Behringwerke, Marburg, Germany) fluorescein isothiocyanate (FITC) labelled goat anti-rabbit gamma globulin (antibody concentration 1·2 mg/ml, FITC to protein ratio 2·5 mol, working dilution 1/20).

The slides were again rinsed twice and washed for 2 min in PBS, mounted with glycerine and examined with a Zeiss Standard microscope equipped with a fluorescent light incident condenser IV F with epi-illumination from an Osram HBO 50W/OC lamp, KP 490/500 Filter for FITC fluorescence and photographed with Kodak Tri X panchromatic film (black and white) and Kodak Ektachrome 160 Tungsten professional (Colour). The same sequence was performed with 'normal' rabbit sera as a control series.

RESULTS

With this previously described, well defined culture system, over 90% of the human skin explants produce a visible epidermal cell outgrowth between days 9–20 in culture. Details of the

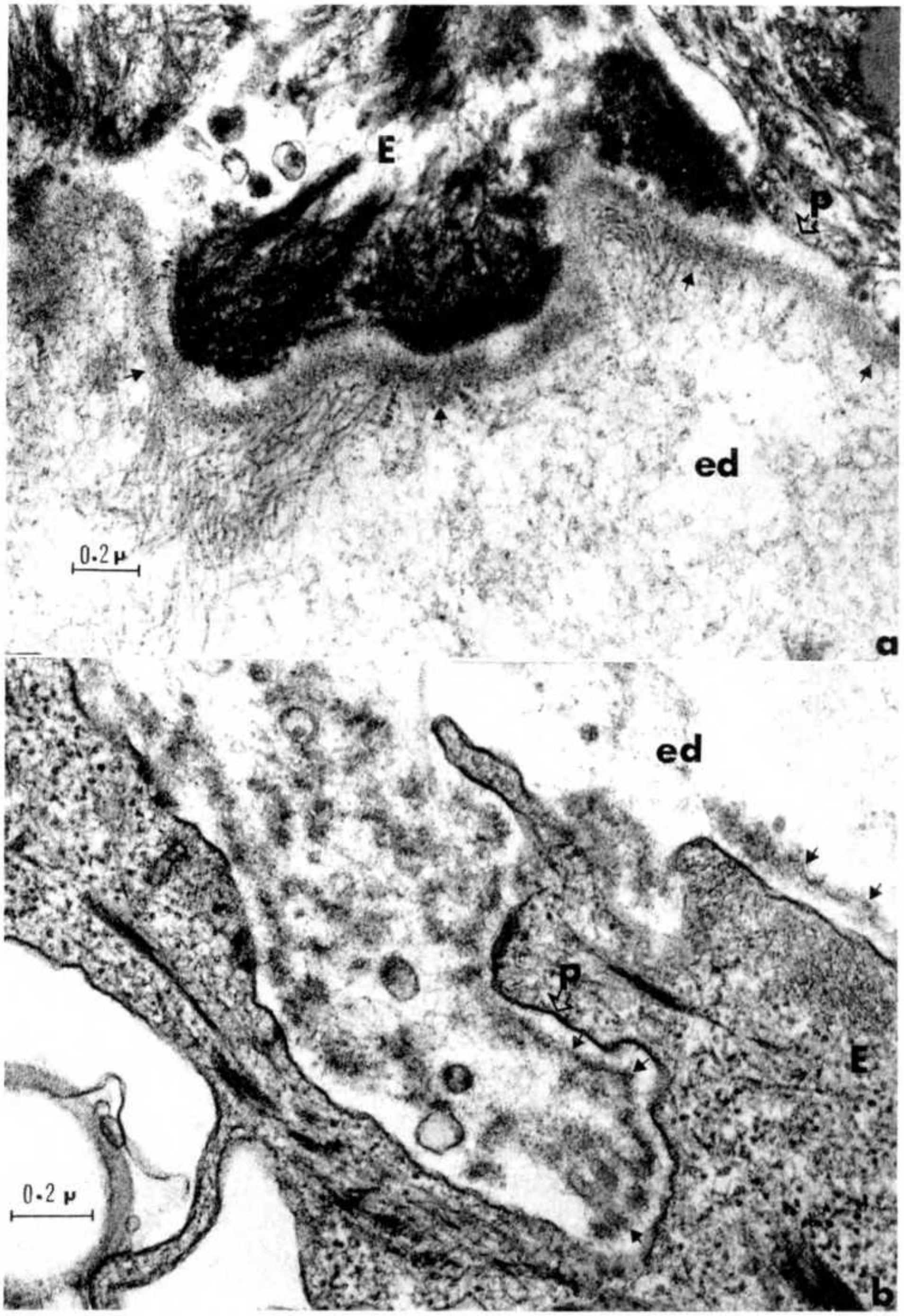

FIGURE 3.(a) Electron micrograph of the original dermo–epidermal junction of an explant taken from culture on Day 23 showing an intact basal lamina. (b) Electron micrograph of the newly-formed dermo-epibolic junction (dej) created by epidermal cells migrating around and under the explant's dermis. E = epidermal cell; P = plasma membrane of epidermal cell; ed = explant dermis; the small solid arrows are pointing to the basal lamina.

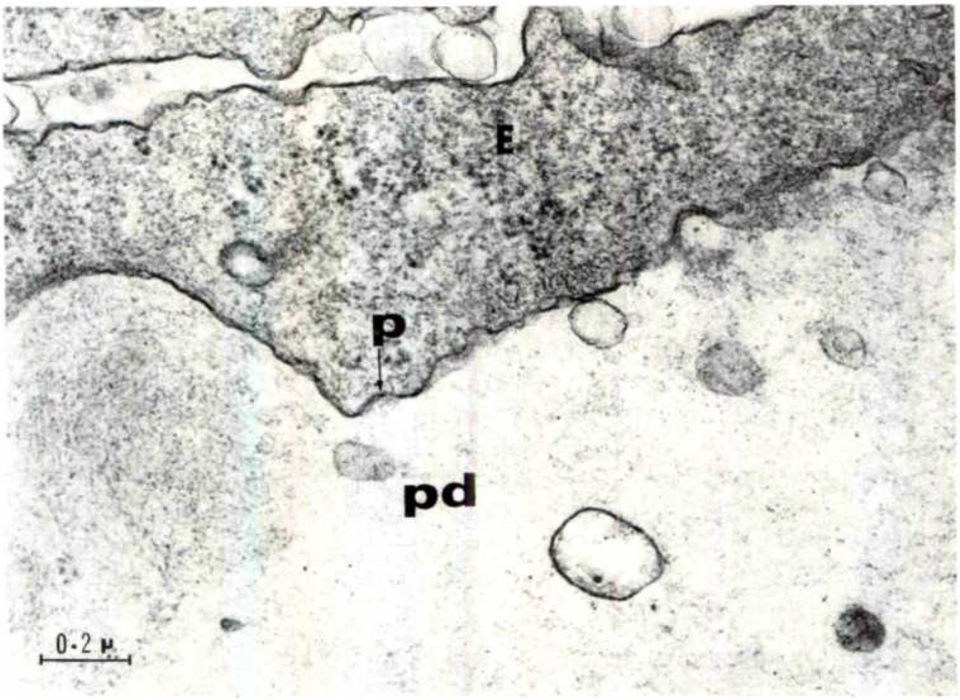

FIGURE 4. Electron micrograph of the outgrowth pig dermal junction from a Day 10 specimen showing an absence of basal lamina formation. P = plasma membrane of epidermal cell; pd = pig dermis; E = epidermal cell.

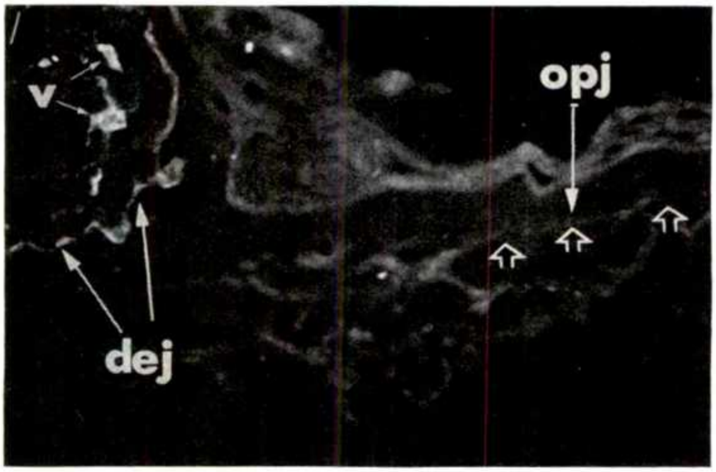

FIGURE 5. A Day 17 explant-outgrowth unit stained by IIF techniques with anti-Type IV collagen antibody. Type IV collagen is present along the dermo-epibolic junction (dej) and around dermal blood vessels (v) in the explant (ex). Type IV collagen is notably absent from the outgrowth pig dermal junction (opj and the hollow arrows) (× 250).

characteristics of this system are published elsewhere (Freeman *et al.*, 1974; Freeman *et al.*, 1976; Woodley *et al.*, 1979; Pruniéras, 1979a, b; Régnier, Pruniéras & Woodley, 1979). With the electron microscope, a somewhat discontinuous basal lamina was found below the epidermal cells of the explant's original dermo-epidermal junction (Fig. 3a). Further, a newly-formed and definitely discontinuous basal lamina was seen with the electron microscope beneath the epidermal cells that had migrated around and under the explant's dermis, to form epiboly and create the so-called 'Dermo-epibolic junction' (Fig. 3b).

In none of the outgrowth pig dermal junctions examined, regardless of the day taken from culture, was a basal lamina formed by electron microscopic criteria (Fig. 4). Specimens taken from culture on Days 10, 17 and 22 and subjected to indirect immunofluorescent staining with anti-Type IV collagen antibody revealed bright linear fluorescence of the DEJ of the explant. A similar fluorescent band was seen at the dermo–epibolic junction created by the epidermal cells that have migrated around and under the explant's dermis (Fig. 5). However, the outgrowth pig dermal junction (created by epidermal cells migrating away from the explant and onto the dead pig dermal substrate) was completely negative in all of the specimens examined (Fig. 5). In this system the explant itself served as a positive control for the reaction. Except for the expected fluorescence noted around blood vessels, the dermis was also negative. In the control series with normal rabbit sera substituted in place of anti-Type IV collagen antibody, linear fluorescent bands were not seen at the DEJ of the explant, the dermo–epibolic junction or the outgrowth pig dermal junction.

DISCUSSION

Briggaman *et al.* (1971) separated human epidermis from its dermis with cold trypsinization, freeze-thawed the dermis ten times to render it non-viable, and then recombined the epidermis onto the inverted killed dermis. That is, the epidermis was recombined to the side of the dermis opposite the side containing the basal lamina. These recombinants were then placed on a chick chorioallantoic membrane at the junction between two vessels. After 2–4 days in culture, a new basal lamina was formed below the epidermal cells within the non-viable dermis. This provided evidence that epidermal cells in culture could synthesize basal lamina (defined by the electron microscope).

Human organ culture studies by Sarkany & Gaylarde (1970) showed that a newly-formed basal lamina appeared beneath the epiboly that migrated around the explant's dermis. At first, this finding could not be confirmed by Marks, Bhogal & Dawber (1972). Later, however, further studies by Marks *et al.* (1975), using a human skin explant system, showed that in two out of nine explants there were 'fragments of whispy electron dense material' beneath the migrating cells and an 'expansion' and 'fuzziness of the basal lamina at its termination at the point of origin of the migrating epidermal cells'. It was thought that this material may correspond to the basal lamina.

In the recent work by Hintner *et al.* (1979) using an explant system similar to Marks *et al.* (1975) but culturing at 33°C, a basal lamina as defined by IIF with an anti-Type IV collagen antibody was found beneath the migrating epidermis after a lag period of 5 days from the appearance of lamina lucida antigens. While this sequence of events is contrary to the organ culture studies of Brickman *et al.* (1977) in which the basal lamina appeared 2 days *before* the appearance of the BP antigen, these authors concluded that both 'lamina lucida antigens and basal lamina antigens may be of epidermal origin but do differ greatly in rate and metabolic requirements for synthesis'.

Stenn, Madri & Roll (1979), using a mouse explant system, did not find Type IV collagen under the migrating epidermis. Rather, it was confined to the explant itself, while the migrating epidermis (epiboly) was found to produce AB_2 (or Type V) collagen.

In the culture system studied here, there are actually three different dermo–epidermal junctions

DEJ): (1) The original DEJ of the explant itself. (2) The dermo–epibolic junction created by cultured cells migrating around and under the explant's dermis (so-called 'epiboly'). (3) The epidermal outgrowth pig dermal junction created by the cultured epidermal cells that migrate away from the explant and are juxtaposed to non-living pig dermal substrate.

Thus, in two of the dermo–epidermal junctions in the system, both components of the junction are living (i.e. the explant's DEJ and the dermo–epibolic junction). In the other one, the outgrowth pig dermal junction, one component is dead (the non-viable pig dermis). In accordance with Sarkany & Gaylarde (1970) and Marks *et al.* (1975), basal lamina was found ultrastructurally at both the explant's original dermo–epidermal junction and the newly-formed dermo–epibolic junction created by epidermal cells migrating around and under the explant's dermis (Fig. 3a and b). The ultrastructural appearance of the basal lamina at both of these locations was somewhat discontinuous, especially at the dermo–epibolic junction, but definitely present.

In accordance with Hintner *et al.* (1979), Type IV collagen in a linear distribution was found at the original DEJ of the explant as well as the dermo–epibolic junction created by the cells migrating around and under the explant's dermis (epiboly).

However, no basal lamina elements, whether defined by the electron microscope or by IIF against anti-Type IV collagen antibody, were present beneath the outgrowth epidermal cells that migrated onto and were juxtaposed to the dead pig dermal substrate; what we have termed the outgrowth pig dermal junction (Figs 4 and 5).

One discernible difference between those culture systems that produce basal lamina and type IV collagen, and the epidermal outgrowth cells used in this study is the element of living material and its position within the system. For purposes of this discussion, 'living material' is defined as 'material that contains living cells'. In the explant studies where basal lamina material was found to be synthesized, the epiboly cells were juxtaposed to living dermis, and the newly formed basal lamina or Type IV collagen was made at this interface. In the recombinant studies of Briggaman *et al.* (1971), the specimens were essentially grafted onto a living chorioallantoic membrane, well vascularized by the living embryo with embryonic, nucleated red blood cells present in the dermal vessels.

In the study reported here, the outgrowth cultured cells are juxtaposed to dead, non-viable substrate devoid of living cells. In addition to the migrating epidermal cells, the only other living material in this system is a small amount of papillary dermis within the explant itself which is markedly deranged and at considerable distance from the major portion of the outgrowth (Fig. 1).

Since the epidermal outgrowth cells in this system have been shown to be capable of stratifying into a multilayered structure, keratinizing and producing both pemphigoid and pemphigus antigens, it may be that some factor (or factors) is missing from the outgrowth that is uniquely required for these other products. This factor may signal the epidermal cell to produce basal lamina, and its origin may be an ephemeral serum component or another cell. If the latter were the case this would be an example of cell to cell communication at the level of the skin.

After finding no basal lamina beneath the epidermal outgrowth of this system, we wondered if there might be something about the texture of the pig dermis that would prevent its synthesis or if the cells could be producing basal lamina material but in a disorganized or soluble fashion that would not be detected with the electron microscope. However, with the negative staining of the outgrowth pig dermal junction and no unusual staining of the pig dermis with anti-Type IV collagen antibody, we doubt that this is the case.

Further work on the possibility that some factor, ratio of collagen (Type I or III) in the substrate, or some form of cell to cell communication is required for the synthesis of basal lamina is currently underway in our laboratory.

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Pharmacology and Treatment

Treatment of hereditary angio-oedema by low dose attenuated androgens: disassociation of clinical response from levels of C1 esterase inhibitor and C4

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SUMMARY

Nine patients with hereditary angio-oedema (H.A.E.) with a deficiency of C1 esterase inhibitor (C$\bar{1}$ INH), have been treated with low dose attenuated androgenic drugs, with complete control of their disease. The C$\bar{1}$ esterase inhibitor and C4 levels became normal in only one patient. It is concluded that H.A.E. can be controlled in the absence of correction of the reduced levels of C1 INH and C4. The mode of action of these drugs appears to be more complex than is generally realized.

Hereditary angio-oedema (H.A.E.) is inherited as an autosomal dominant. Attacks consist of bouts of non-itchy swellings which may involve the skin and mucous membranes including the larynx and intestine. The swellings may last for several days, and an attack is often precipitated by trauma. Intestinal involvement may lead to attacks of abdominal colic which may be severe and may cause intussusception. Laryngeal involvement may be fatal.

H.A.E. is associated with a deficiency of C1 esterase inhibitor (C$\bar{1}$ INH). This protein is an α-neuroaminoglycoprotein of mol. wt. about 90,000. In 85% of pedigrees there is a deficiency of synthesis of C$\bar{1}$ INH and in 15% it is produced in normal or increased amounts, but is functionally inactive (Rosen *et al.*, 1971).

In the presence of tissue injury, C1 is activated to C$\bar{1}$. If C$\bar{1}$ INH is reduced or is functionally inactive, C$\bar{1}$ cleaves its natural substrates C4 and C2. In the process of formation of C4·2, a vasoactive kinin-like fragment (C2 kinin) is formed.

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The treatment of the acute attack is with fresh frozen plasma or an infusion of the purified C$\bar{1}$ INH. Until recently, anti-esterases have been the main prophylactic treatment, and in many cases reduce the frequency and severity of attacks.

There have been several reports of the ability of weakly androgenic anabolic steroids to prevent attacks (Gelfand *et al.*, 1976; Sheffer, Fearson & Austen, 1977; Pitts *et al.*, 1978; Saihan & Warin, 1978; Tappeiner *et al.*, 1979). The drugs used have included danazol, methandienone and stanozolol. It has been shown that in the dosages usually recommended, the plasma C$\bar{1}$ INH and C4 levels rise towards normal. It has therefore been assumed that correction of the underlying biochemical abnormalities underlies the action of these drugs.

The purpose of this study was to reduce the dose of the attenuated androgenic drugs to the lowest level possible, commensurate with control of the disease. This study has also provided an opportunity to examine the relationship between disease control and plasma levels of C$\bar{1}$ INH and C4.

PATIENTS AND METHODS

Nine patients (five males, four females) with H.A.E. have been studied; mean age 40 (25–69). There was a family history of H.A.E. in seven patients. The mean frequency of attacks was every 6 weeks (range 1 per week to 1 per 8 weeks). Eight had had episodes of abdominal pain and all nine had had attacks of laryngeal oedema. All nine patients had a low C$\bar{1}$ INH level before treatment; mean 0·07 g/l (range 0–0·16, normal 0·18–0·26). At the time that they were started on attenuated androgens, and throughout the study, they were not on any other drugs. C$\bar{1}$ INH was estimated immunochemically by the single radial immuno-diffusion assay of Fahey & McKelvey (1965). C4 was estimated by a single radial immuno-diffusion assay using monospecific antibody to C4.

Three patients were treated with danazol 200 mg twice daily, later reduced gradually to 200 mg on alternate days. Two patients were treated with methandienone 5 mg twice daily, later reduced to 2·5 mg daily in one patient and on alternate days in the other, and four patients were treated with stanozolol 5 mg daily, later reduced to 5 mg every 2–10 days.

RESULTS

Response to treatment

The attacks of angio-oedema were prevented in all patients while on high dose attenuated androgens. The dosage was gradually reduced over 12–18 months.

Danazol. One patient had several attacks of abdominal pain and one of laryngeal spasm while on 200 mg on alternate days. Attacks were prevented when the dose was increased to 200 mg per day. At the time of the first of these minor attacks his C$\bar{1}$ INH was 0·06 g/l and C4 was 0·06 g/l.

A further patient (A.K.) had a minor attack of swellings while on danazol 200 mg on alternate days. No biochemical findings are available at the time of the attack, but she increased her danazol to 200 mg daily and had no further episodes. At the time of her next visit to hospital, the C$\bar{1}$ INH was 0·27 g/l and C4 0·32 g/l.

Methandienone. One patient had minor swellings 5 weeks after the dose was reduced to 2·5 mg on alternate days. After the dose was increased to 2·5 mg per day, no further attacks occurred. The C$\bar{1}$ INH at the time of attacks was 0·09 g/l.

The other patient has not had his dose reduced below 2·5 mg per day.

Stanozolol. Four patients were on stanozolol 5 mg every 2–10 days and none has had any relapse. The dose has not been reduced further to determine the minimum dose required to control the attacks.

Effect on biochemical findings

C$\bar{1}$ INH (*Fig. 1*). All nine patients had a low C$\bar{1}$ INH level before treatment (mean 0·07 g/l, range 0–0·16, normal 0·18–0·26). In A.K., the C$\bar{1}$ INH became normal after treatment and has remained normal for 12 months. She is well controlled on danazol 200 mg on alternate days. In two further patients, the C$\bar{1}$ INH levels became normal and yet were reduced to pre-treatment levels by 18 months. The remaining patients did not show an appreciable rise on high dosages and in the six patients who have now been on treatment for 18 months, the mean C$\bar{1}$ INH level was 0·05 g/l (range 0–0·10, normal 0·18–0·26).

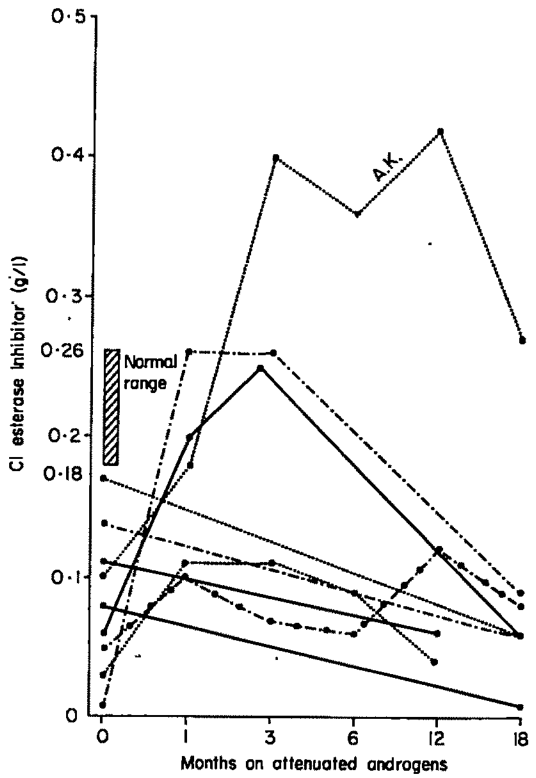

FIGURE 1. C$\bar{1}$ INH in nine patients with H.A.E. before and during treatment with attenuated androgens.

C4 (*Fig. 2*). The C4 level was estimated in seven patients, six of whom showed a low C4 before treatment; mean 0·10 g/l (range 0·01–0·15, normal 0·23–0·75). In none of these patients did the C4 level rise to normal and in the five patients in whom C4 was measured after 18 months' treatment, the mean level was 0·10 g/l (range 0–0·12, normal 0·23–0·75). In A.K. the C4 level was normal before treatment and remained normal for the next 12 months.

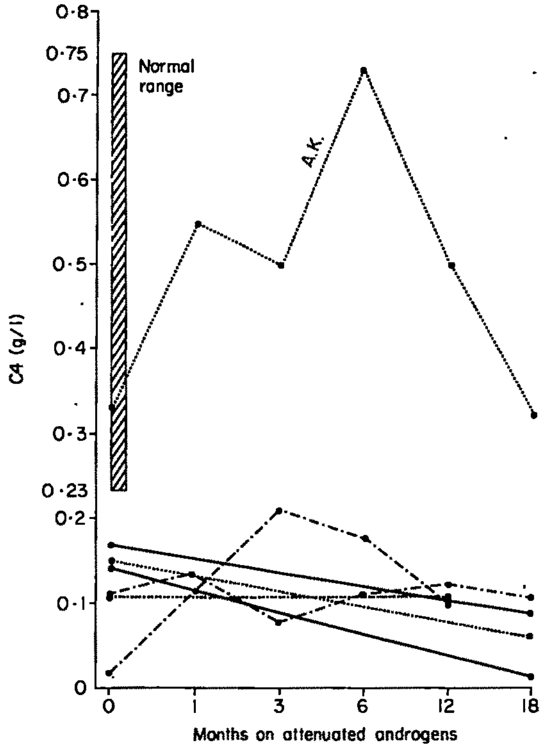

FIGURE 2. C4 levels in seven patients with H.A.E. before and during treatment with attenuated androgens.

DISCUSSION

The recent introduction of androgenic drugs for the treatment of H.A.E. has been a significant advance. The drugs used are attenuated androgens with anabolic but little masculinizing activity. The dosages that are in general use have been demonstrated to elevate the C$\bar{1}$ INH and C4 levels towards normal, and this has been their supposed mode of action (Gelfand *et al.*, 1976; Pitts *et al.*, 1978). However, Lagrue *et al.* (1978) found that low dose danazol or methandrostenolone could control the attacks with a barely detectable effect on the biochemical abnormalities.

The data presented show that if the dose of attenuated androgen is reduced to a low level, the levels of C$\bar{1}$ INH and C4, which may be corrected initially, return to pre-treatment levels in most cases, and yet the attacks are still prevented. The mode of action is not known, but it is possible that these drugs interfere with activation of C1. Alternatively, since these drugs increase protein synthesis by the liver, they will increase the synthesis of other inhibitor proteins which may compensate for the lack of the C$\bar{1}$ INH.

The use of attenuated androgens in children and pregnant women is not without hazard, and so anti-esterase drugs still have a place. A recent report of a child with H.A.E. who continued to grow while on danazol is encouraging (Tappeiner *et al.*, 1979). It seems likely that in the small doses required, this drug may be used in children. The treatment of choice for young women not on an effective contraceptive is still anti-esterase therapy.

In conclusion, the doses of attenuated androgens required to control H.A.E. appear to be much lower than has been recommended previously, and it does not seem to be necessary to control the C1̄ INH and C4 levels in order to control the disease. We would now recommend the following minimum doses that appear necessary to prevent attacks: danazol: 200 mg per day; methandienone: 2·5 mg per day; stanozolol: 5 mg twice a week.

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Psoriatic arthritis treated with oral zinc sulphate

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SUMMARY

Twenty-four patients suffering from psoriatic arthritis participated in a double blind cross-over trial of peroral zinc sulphate versus placebo (Trial I). Eleven patients continued an open trial of peroral zinc sulphate for an additional 24 weeks (Trial II). Remission was assessed by the disappearance of symptoms (overall condition, morning stiffness, functional capacity of the joints and joint pains), and signs (mobility and swelling of the joints). Reduction of joint pains as well as increase of mobility and decrease of swelling of several joints were observed. The clinical signs of reduced inflammation were accompanied biochemically by reduction of serum immunoglobulins and an increase of serum albumin. The need for analgesics was diminished. Severe side-effects and changes in the psoriatic skin involvement were not seen. Oral zinc sulphate seems to be valuable in the treatment of psoriatic arthritis.

Zinc exerts various biological functions, including an ability to reduce inflammatory processes through inhibition of prostaglandin synthesis, macrophage function, and the complement system (Nugteren, Beerthuis & Van Dorp, 1966; Karl, Chvapil & Zukoski, 1973; Yamamoto & Takahashi, 1975), and possibly a stabilization of lysosomal membranes (Chvapil, Ryan & Zukoski, 1972).

Patients with psoriasis and rheumatoid arthritis (RA) may have disturbances of zinc metabolism, but an unequivocal pattern is not evident (Greaves & Boyde, 1967; Vorhees *et al.*, 1969; Niedermeier & Griggs, 1971; Tsambaos & Orfanos, 1977; Buneaux *et al.*, 1978). Simkin (1976) and later Menkes, Job & Delbarre (1978) have reported a beneficial effect of treatment with zinc sulphate in patients with RA.

These results and the reported anti-inflammatory effect of zinc prompted us to examine the effect of zinc sulphate on psoriatic arthritis. The low toxicity of the drug and the limited ways of treating psoriatic arthritis further added to the indications for the trial.

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MATERIAL AND METHODS

Forty consecutive patients with psoriatic arthritis seen at the Department of Dermatology between 1975 and 1977 were invited to participate in the trial; twenty-nine patients agreed.

Only psoriatics with negative tests for rheumatoid factors, LE-cells, and antinuclear antibodies were included. The diagnosis of arthritis was based on persistent arthralgia with either functional impairment or measurable swelling of a joint. Two or more joints should be involved, at least one being a hand or finger joint. Five patients were excluded during the study, due to reconsideration of the diagnosis in one and inability to attend the follow-up visits in four patients. The final material consisted of twenty-four patients, five males and nineteen females, with a mean age of 50 years (range 18–76 years). The mean duration of psoriasis was 26 years (range 4–57 years), and of joint involvement 7 years (range 3/4–22 years). The mean extent of skin involvement was 11% (range 2–85%). In all but three patients the involvement of the skin preceded the psoriatic arthritis.

The patients' overall condition, joint pains, number of involved joints, and the functional capacity of the joints before they entered the study are shown in Table 1.

TABLE 1. Assessments of symptoms by twenty-four patients with psoriatic arthritis before treatment

Grade	Joint pains	Functional capacity of the joints	Overall condition	Number of joints involved†
1	0	2	0	1
2	4	2	5	5
3	11	15	15	2
4	7	5	4	4
5	2	0	0	12

* The figures refer to the number of patients affected by symptoms, graded from 1 to 5, Grade 1 representing normal and Grade 5 a very disabling condition.

† Grade 1 = less than 5 joints; Grade 2 = 5–10 joints; Grade 3 = 11–15 joints; Grade 4 = 16–20 joints, Grade 5 = more than 20 joints.

Five patients were on a constant methotrexate (MTX) regimen during the study.

Analgesics such as salicylates and dextropropoxyphene were allowed freely, but the mean daily intake was recorded at each visit as well as the changes of topical treatment, extent and activity of skin involvement and of bodyweight.

A double-blind crossover trial of 12 weeks (Trial I) comprising 6 weeks of zinc sulphate treatment (tablets of 220 mg three times daily) and 6 weeks of placebo (tablets three times daily) was scheduled. The patients were randomly allocated to receive either zinc sulphate or placebo first.

Following Trial I the twelve patients who entered the study first continued to receive zinc sulphate in an open study of 6 months' duration (Trial II). Eleven patients completed Trial II, one patient being excluded due to changes in MTX treatment.

The patients were examined every third week during Trial I, and those included in Trial II had a final examination at the end of the study. At each visit the number of affected joints and the duration of morning stiffness as evaluated by the patients were recorded. The patients' overall condition, their pain and functional capacity of the joints were recorded. Side-effects due to the treatment were noted.

In order to reduce the number of parameters and to obtain comparable results, only wrists and finger joints were examined. Joint movements were determined by a protractor, joint circumferences were measured with rings, grip strength between fingers and that of each hand was examined with a strain-gauge apparatus, and the individual finger strength by a dynanometer (Asmussen, Heebøll-Nielsen & Molbech, 1959).

At each visit serum concentrations of zinc, iron, transferrin, creatinine, albumin, IgG, IgA, IgM, IgE, alkaline phosphatase, SGOT, uric acid, antinuclear antibodies and complement C3 and C4 together with ESR, haematocrit, white blood cell and platelet counts were measured. The urine was screened for protein and formed elements.

The results after 6 weeks of zinc sulphate treatment were compared to the results after 6 weeks of placebo regardless of whether zinc had been given early or late in the study. The results following Trial II were compared to the results of the initial evaluation. The Wilcoxon rank sum test was used for statistics.

RESULTS

The treatment was well tolerated. Slight dyspepsia (nausea, epigastric and diffuse abdominal dull pains, loose bowels, constipation) of 1–2 weeks duration was recorded by seven patients—five of whom were on zinc treatment and two on placebo. The difference is not statistically significant. No additional treatment of these minor disturbances was necessary.

Patient assessments

This included overall condition, morning stiffness, joint pains and functional capacity of the joints. During the zinc sulphate treatment of Trial I some remission in all assessments was noticed, but only the joint pains were significantly reduced ($P<0{\cdot}02$) (Table 2).

During Trial II this reduction was still apparent ($P<0{\cdot}02$) but, furthermore, a significant reduction of the morning stiffness ($P<0{\cdot}05$) and an improvement of the overall condition ($P<0{\cdot}01$) were demonstrated (Table 2). The functional capacity of the joints was not significantly improved.

The mean daily intake of analgesics was reduced during the zinc sulphate treatment in both trials, being most pronounced in Trial II ($P<0{\cdot}01$).

TABLE 2. Variation of joint symptoms in patients with psoriatic arthritis after 6 weeks (Trial I) and 6 months (Trial II) of zinc sulphate treatment*

		Patients' assessments			Motility				Swelling		
		Morning stiffness	Overall condition	Joint pains	FW	MP	PIP	DIP	MP	PIP	DIP
Trial I	Improved	10	6	9	14	14	12	14	9	10	10
(double blind	Unaltered	9	16	14	3	2	3	1	9	6	3
controlled trial)	Worsened	5	2	1	7	8	9	9	6	8	6
(n = 24)	*P*<	n.s.	n.s.	0·02	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Trial II	Improved	9	8	9	11	7	7	9	7	8	9
(open trial)	Unaltered	1	3	1	0	0	0	0	3	0	1
	Worsened	1	0	1	0	4	4	2	1	3	1
(n = 11)	*P*<	0·05	0·01	0·02	0·01	0·05	n.s.	0·01	0·05	n.s.	0·01

* FW = flexion of wrists. MP = metacarpophalangeal joints. PIP = proximal interphalangeal joints. DIP = distal interphalangeal joints.

Clinical evaluation

Zinc sulphate treatment caused an increase in motility and a decrease in swelling of most of the tested joints during both trials, however only to a significant level during Trial II ($P<0{\cdot}05$) (Table 2). Measurable changes in 'grip' and 'finger' strengths were not seen in Trials I or II.

The intensity and the degree of the psoriatic skin involvement was unaltered in both trials.

Blood chemistry

During zinc sulphate treatment the normal pretreatment serum zinc concentrations increased significantly ($P<0{\cdot}02$). The immunoglobulin values decreased, especially IgA ($P<0{\cdot}05$) and IgM ($P<0{\cdot}02$), and the serum albumin concentrations increased ($P<0{\cdot}05$). All other laboratory values apart from a slightly elevated ESR, unaltered during treatment, were within normal range.

DISCUSSION

The diagnostic criteria of psoriatic arthritis in this study—seronegative arthritis in psoriatics—are in accordance with those of others (Wright, 1959; Baker, 1966). Effective treatment of the arthritis in these patients is often difficult, if drugs with possible severe side effects such as cytostatics, corticosteroids, gold, and antimalarials are to be avoided (Copeman, 1967; Baker & Ryan, 1968; Oppolzer, Diem & Wolf, 1976; Nolting & Kastrup, 1978).

Peroral zinc sulphate treatment as examined in this study was followed by an improvement of the joint condition based on the patients' own assessments as well as on the objective results. This improvement together with the reduced consumption of analgesics was, however, most pronounced in Trial II (Table 2). This difference between Trials I and II is possibly due to a slow effect of zinc sulphate.

It is unlikely that a placebo effect would persist for 6 months and the condition even improve. The reduction of analgesics further favours a genuine effect of zinc sulphate. The variations in the patients' assessments were followed by quantitatively measurable changes of joint parameters and improvement of laboratory parameters ordinarily connected with inflammatory disease (immunoglobulins, albumin). The reduction of immunoglobulins and increase of albumin coincided with the decrease of disease activity. Zinc sulphate *per se* has not been reported to affect serum protein levels.

Serum zinc values before treatment were within the normal range so the effect of treatment is not explained by an alleviation of an absolute zinc deficiency.

The medication was followed by no serious side-effects, even after 6 months of treatment. No decrease in serum iron, which has been reported (Porter *et al.*, 1977), was seen.

It seems fair to conclude that oral treatment with zinc sulphate might prove valuable in the treatment of psoriatic arthritis.

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Serum levels of 8-methoxypsoralen: difference between two oral methods of administration*

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SUMMARY

Serum levels of 8-methoxypsoralen were determined in six human volunteers after two different methods of oral administration.

8-Methoxypsoralen was given as an aqueous solution and as powder in gelatin capsules. The area under the curve (AUC) values were higher ($P<0.05$) for the solution than for the capsules, indicating a higher bioavailability. Also, the peak serum levels were higher and were reached earlier when given as a solution. Considerable individual variation of the maximum serum levels and the area under the curve values was observed for both methods of administration, but the time required to reach the maximum serum level was more predictable after administration of the solution.

Oral administration of 8-methoxypsoralen (8-MOP) followed by longwave ultraviolet (UV-A) irradiation, about 2 h after ingestion of the drug, has been used for the past few years in the treatment of psoriasis. The usual dose is 0·6 mg 8-MOP/kg. Recently Swanbeck *et al.* (1979) have demonstrated a correlation between the plasma concentration of 8-MOP and photosensitivity. A wide variation of plasma levels of patients treated with 8-MOP powder capsules was reported by Steiner *et al.* (1977). Probably the poor water solubility of this drug causes differences in absorption rate and bioavailability. In this study the bioavailability of two different oral dosage forms has been compared in healthy subjects.

MATERIALS AND METHODS

Dosage forms

8-MOP solution: 40 mg 8-MOP|| was dissolved in a mixture of 10 ml absolute ethanol and 10 ml

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* Based on a poster exhibition presented at the 218th meeting of the Netherlands Association for Dermatology and Venereology, 23 February 1980.

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orange syrup (Netherlands Pharmacopoeia, 6th edn). Just before ingestion the solution was added to about 150 ml orange juice and mixed. 8-MOP remains dissolved in this supersaturated solution for at least 4 h.

8-MOP powder in gelatin capsules: 1 g 8-MOP was dissolved in 3 ml chloroform (Neth.P. 6th edn) and the solution was mixed with 10 g micro-crystalline cellulose (Avicel, pH 102).* The chloroform was evaporated and powder, equivalent to 10·0 mg 8-MOP, was weighed into hard gelatin capsules. In this way a micronized form of 8-MOP was obtained. Microscopic examination showed a particle size ranging from 5 to 50 μm.

Investigational procedure

Six healthy human subjects, one female and five male, with normal liver and renal function tests, ranging in age from 21 to 35 years and in body weight from 63 to 75 kg, participated in this study. Just before ingestion a standardized breakfast was eaten. The latter consisted of one slice of brown bread with margarine and strawberry jam. Then the 8-MOP solution was given, followed by an additional 100 ml orange juice. The capsules (four capsules, each containing 10·0 mg 8-MOP) were given with 100 ml orange juice. Four weeks elapsed between the two tests. No additional food was allowed 10 h before and 4 h after the drug administration. No other drugs were allowed to be taken 1 week before the test. Blood samples were taken just before and at 0·25, 0·50, 0·75, 1, 1·5, 2, 3 and 4 h after administration of the 8-MOP solution and at 0·5, 1, 1·5, 2, 2·5, 3, 4 and 5 h after administration of the 8-MOP capsules.

The serum fractions were separated and stored at —20°C until assayed. Also urine was collected just before ingestion and at 0–2, 2–4, 4–6 and 6–24 h after ingestion. The volume of the urine portions was measured and samples of about 100 ml were stored at —20°C until assayed.

The amount of 8-MOP in serum and urine was determined as described elsewhere (Stolk, 1980). The procedure is based on a single extraction with an organic solvent, chromatography on a reversed phase C18 column and UV detection at 254 nm.

Pharmacokinetic parameters

The area under the serum concentration time curve (AUC) was determined by the trapezoid rule from 0 to 4 h.

RESULTS

Subjective side-effects

About 1 h after administration of the 8-MOP solution Subjects 1, 2, 3 and 4 complained of nausea. Moreover, Subjects 2, 3 and 4 experienced dizziness and sweating. These symptoms disappeared about 2 h later. After administration of the capsules only Subject 4 complained of having the same symptoms, but to a much lesser degree.

Pharmacokinetics

Serum concentration curves for 8-MOP after administration of the solution and the capsules are shown in Fig. 1. It can be seen that, after administration of the solution, earlier and higher maximum serum levels (C_{max}) were observed. The values of AUC ($P<0·05$) and C_{max} ($P<0·05$) were higher after administration of the solution than after administration of the capsules (Table 1).

The mean time required for reaching C_{max} was 1 h for the solution and 2·1 h for the capsules. Table 1 also shows the total amount of unchanged 8-MOP excreted in urine.

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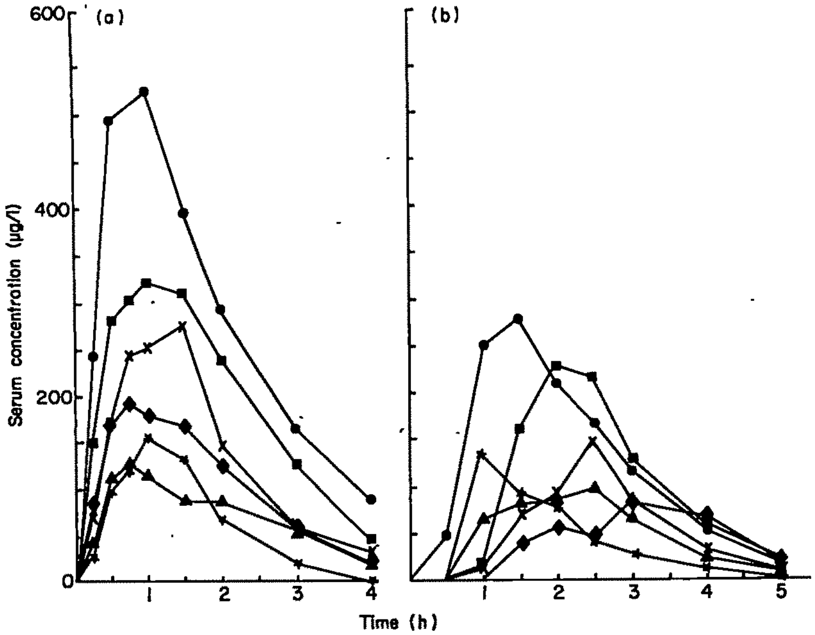

FIGURE 1. Serum levels of 8-MOP, after oral administration as (a) a solution and (b) powder capsules. (●——●) Subject 1; (▲——▲) Subject 2; (■——■) Subject 3; (X——X) Subject 4; (◆——◆) Subject 5; (★——★) Subject 6.

TABLE 1. Maximum serum concentration (C_{max}), time for serum peak concentration (t_{max}), area under the curve (AUC) and excretion in urine for 8-MOP given as a solution and as powder capsules

Subject	C_{max} (µg/l)		t_{max} (h)		AUC (0-4) (µg h L^{-1})		Ratio $\frac{\text{AUC capsules}}{\text{AUC solution}}$	Total 8-MOP excreted in urine (µg)	
	solution	capsule	solution	capsule	solution	capsule		solution	capsule
1	524	278	1	1·5	1129	585	0·52	8·8	8·5
2	127	97	0·75	2·5	286	233	0·81	2·3	3·1
3	321	229	1	2	780	435	0·56	n.d.	3.9
4	275	146	1·5	2·5	534	240	0·45	5·2	2·3
5	193	79	0·75	3	425	163	0·38	5·3	n.d.
6	155	135	1	1	255	200	0·78	4·6	2·8
Mean	266	161	1	2·1	568	309	0·58	5·2	4·1
± S.d.	146	77	0·3	0·7	334	165	0·18	2·3	2·5
Significance level (paired t-test)	$P < 0·05$		$P < 0·05$		$P < 0·05$				

n.d. = not determined.

There was no significant difference in excretion of 8-MOP in urine for the two dosage forms. 8-MOP could be determined in all urine portions collected from 0 to 2 and from 2 to 4 h after ingestion. No unchanged 8-MOP could be detected in urine samples collected from 4 to 6 h and 6 to 24 h, except in urine samples (4–6 h) of Subjects 1 (solution) and 3 (capsules).

DISCUSSION

The bioavailability of 8-MOP is higher after administration as a solution than as capsules. However, for both dosage forms the AUC and the peak serum levels are liable to considerable individual variation. Therefore, other factors besides rate of dissolution must be responsible for the variation. 8-MOP is readily metabolized (Busch *et al.*, 1978). Possibly a first pass effect causes the individual differences in the serum levels. After administration of the capsules, the time required to reach maximum levels of 8-MOP varies very much, but after administration of the solution for all subjects a peak level was achieved in about the same time: 1 h.

If dermatological patients are to be irradiated at the time of the highest 8-MOP level, an 8-MOP solution would be preferable, because the timing of the maximum serum level is more predictable.

However, more subjective side-effects were experienced with the solution than with the capsules. At present we are developing other dosage forms of 8-MOP with the aim of achieving high and predictable serum levels and minimal side-effects.

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Case Reports

A syndrome of various cutaneous pigmented lesions, myxoid neurofibromata and atrial myxoma: the NAME syndrome

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SUMMARY

A patient with profuse cutaneous pigmented lesions, subcutaneous myxoid neurofibromata and atrial myxoma is described. This is the second report of this syndrome, whose importance lies in the possibility of avoiding the potentially lethal consequences of a treatable cardiac tumour by recognition of distinctive cutaneous manifestations.

CASE REPORT

History

M.S. was born spontaneously at full-term (birth weight: 3·27 kg) following an uneventful pregnancy, though his mother was taking the contraceptive pill throughout the first two trimesters. Three flat pigmented lesions were noted at birth on the neck, trunk and thigh, and during the first few weeks of life large numbers of other smaller pigmented lesions appeared; these were always more prominent in the summer months. At the age of 2 years he developed an asymptomatic swelling behind the right ear, which was excised. This was reported histologically to be a myxoid liposarcoma. The following year, two similar lumps were excised from his chin and from the front of the chest, and a further one was removed from behind the right ear at the age of 8 years. These were all similar and considered to be myxoid liposarcomata.

Family History

Both parents were heavily 'freckled', though less so than the patient. His two brothers aged 14 and 12 years were also 'freckled', but not excessively. His mother had red hair and blue eyes. His father and brothers had light-brown hair and all had blue eyes. His mother had a hypochromic naevus over the right scapula.

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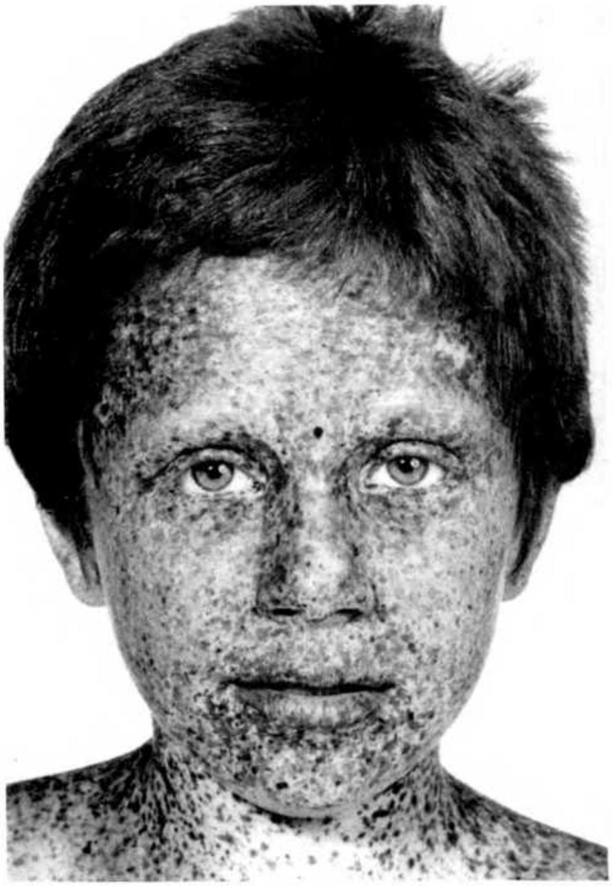

FIGURE 1. Face, showing vast numbers of macular pigmented lesions of various hues. Note the involvement of the lips.

Examination

He was seen by us at the age of 10 years. His weight was 30·1 kg and his height 134·5 cm (both on the 50th centile). His eyes were blue and his hair was of a distinctive rust-red colour. In the skin, a profusion of pigmented lesions was apparent (Figs 1–3). The majority were small macules up to 1 cm in diameter, distributed principally on the face, neck, shoulders, upper chest and back, the extensor aspects of the arms, the dorsa of the hands, the anterior thighs and the popliteal fossae. The majority of these lesions were pale brown and they tended to become confluent in the areas of their greatest density. Interspersed in the same distribution were darker brown macules of a similar size. Approximately twelve typical, domed blue naevi were present at various sites on the trunk and limbs, and three larger pigmented macules up to 2 cm in diameter were present, one in the left anterior triangle of the neck, one on the right side of the abdomen and the other just below the right buttock; these were the lesions present at birth. There were no axillary lesions, but several pigmented macules were scattered on the palmar surfaces of the fingers. The texture of the skin was normal; in particular there was no scarring or atrophy. Pigmented macules were present on the mucosa of the lips and a

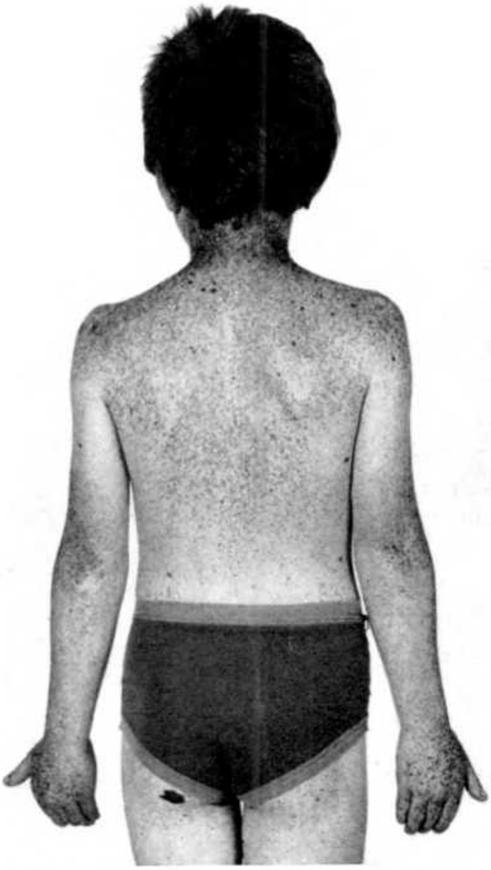

FIGURE 2. Rear view of the patient. The larger pigmented macule in the left gluteal fold was one of three lesions present at birth.

single small pigmented macule within the mouth on the right buccal mucosa. One small area of macular pigmentation was present on the conjunctiva of the right eye. Fundal examination was normal.

Although mitral systolic and diastolic murmurs were heard on some occasions, these were not apparent at the initial examination and showed marked variation from day to day, and with posture. Physical examination including sexual development was otherwise normal.

Investigations

Full blood-count: haemoglobin 13·2 g/dl, white cell count 8·5 × 10^9/l with a normal differential. E.S.R. 43 mm/h. Chest X-ray showed slight enlargement of left atrium and prominent upper lobe pulmonary vasculature. E.C.G. showed evidence of early left atrial hypertrophy (P 'mitrale') but was otherwise normal. Echocardiogram (Fig. 4a) indicated the presence of a large left atrial tumour. Right heart catheterization revealed mild pulmonary hypertension (PA pressure 45/20 mmHg). Right ventricular angiogram was normal, but follow-through to the left atrium outlined a large, mobile atrial tumour. Audiometry tests were normal. D.N.A. excision and post-replication repair studies (Dr F.B. Giannelli) were normal. Monochromator tests (Dr J. Hawk) were also normal.

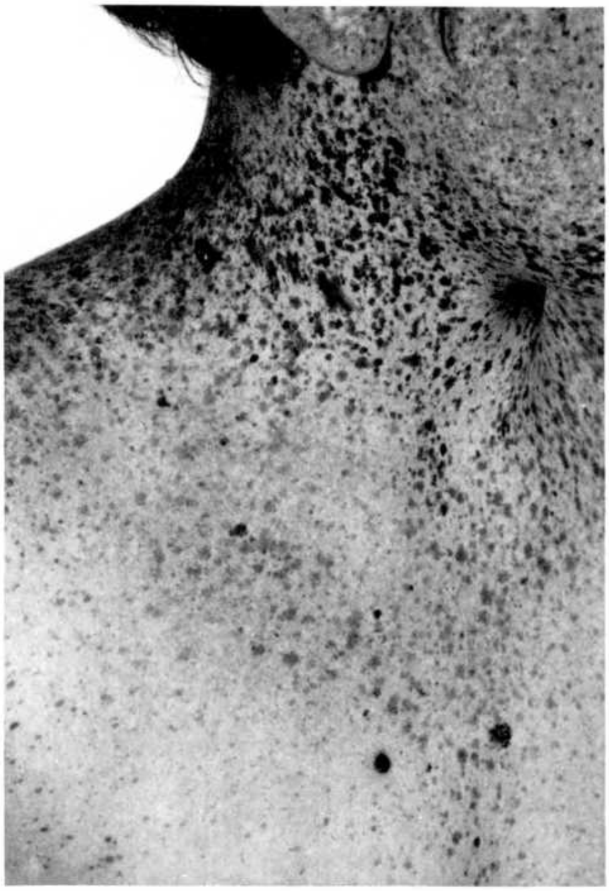

FIGURE 3. The right side of the neck and upper chest. Note the domed blue naevi.

Progress

At operation, a friable and gelatinous mass was found in the left atrium originating from the interatrial septum. Part of the tumour extended through the foramen ovale into the right atrium, and a smaller, separate tumour was attached to the free wall of the right atrium opposite the foramen. The tumours were excised and the interatrial septum was repaired with Dacron. Post-operative progress was uneventful. Subsequent echocardiograms showed no evidence of residual atrial tumour up to 1 year after the operation (Fig. 4b).

A further subcutaneous nodule has recently appeared behind the left ear. This was about 5 mm in diameter, spherical and freely mobile; it has now been excised.

Histology

Pigmented skin lesions. Several of the widespread pigmented macules were biopsied. These proved to be ephelides, the paler lesions showing increase of melanin pigmentation restricted to the basal layer, while darker lesions showed more intense melanization of the basal and lower spinous layers, but there was no epidermal hyperplasia or increase in melanocyte numbers (Fig. 5). A naevocytic naevus was identified histologically but none of the lesions biopsied could be classified as a lentigo.

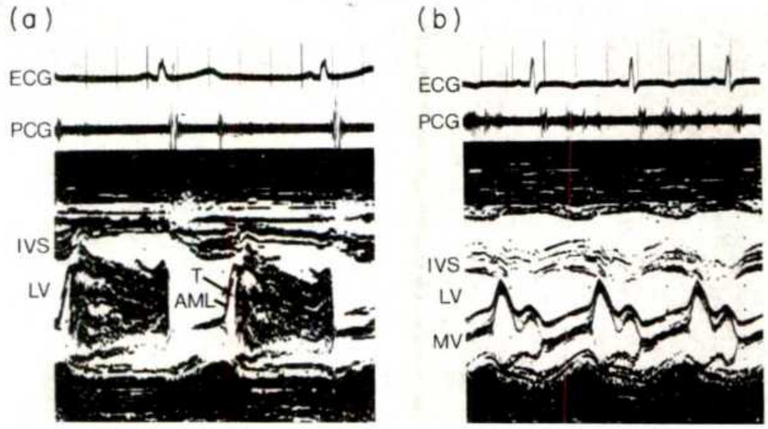

FIGURE 4. Echocardiograms with simultaneous electrocardiogram (ECG) and phonocardiogram (PCG). (a) Pre-operative record. The ultrasound beam passes through the left venticle (LV) at the level of the mitral valve. Echoes from the interventricular septum (IVS) are seen bordering the LV anteriorly (top of record) and echoes from the pleura and lung are seen posteriorly (bottom of record). During diastole the space between the mitral leaflets is filled by dense echoes arising from the tumour (T), and the normal M-shaped motion of the anterior mitral leaflet (AML) in diastole is blunted. (b) Post-operative recording across the LV at the same level. The mitral valve (MV) now moves normally and no abnormal echoes appear between its leaflets in diastole. The PCG now shows a pericardial friction rub.

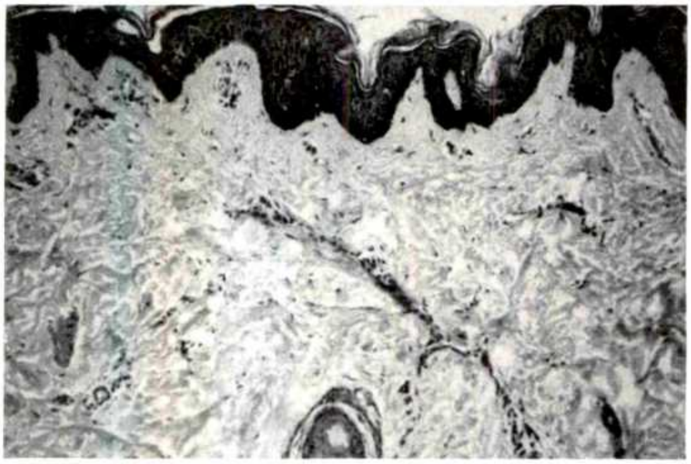

FIGURE 5. Histopathology of acquired dark brown macular lesion. There is prominent melanin pigmentation of basal and suprabasal layers of the epidermis in the absence of numerical increase of melanocytes (H & E, × 120).

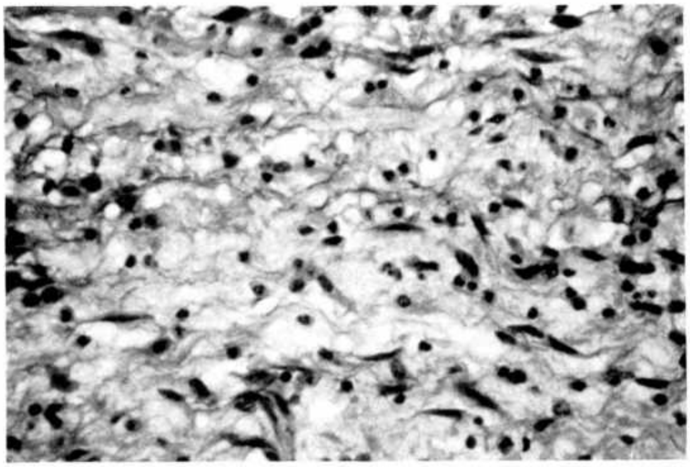

FIGURE 6. Myxoid neurofibroma. Stellate and bipolar spindle cells give rise to wavy fibrillar processes. Numerous polymorphonuclear leukocytes are present (H & E, × 200).

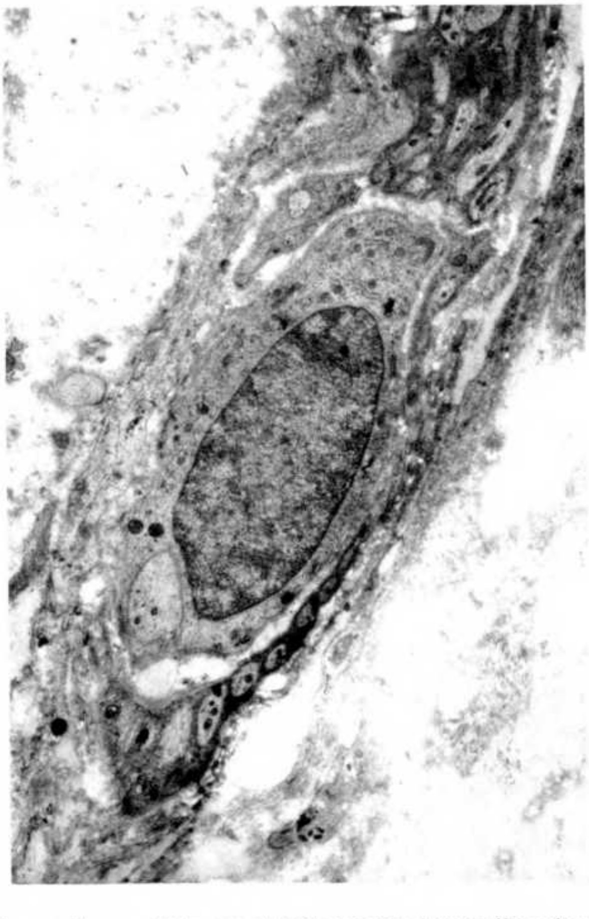

FIGURE 7. Myxoid neurofibroma. Schwann cell with associated unmyelinated neurites embedded in featureless ground substance (E.M. × 17,000).

The domed black papular lesions were typical blue naevi. One of the excised lesions showed the co-existence of a blue naevus with an overlying naevocytic naevus. Giant melanosomes were not identified by light microscopy or ultrastructurally.

Subcutaneous tumours. The histology of the three tumours previously removed was reviewed, and compared with the recent tumour from behind the ear. All showed essentially the same features. The tumours consisted of circumscribed dermal nodules with a background fibrillary stroma which in

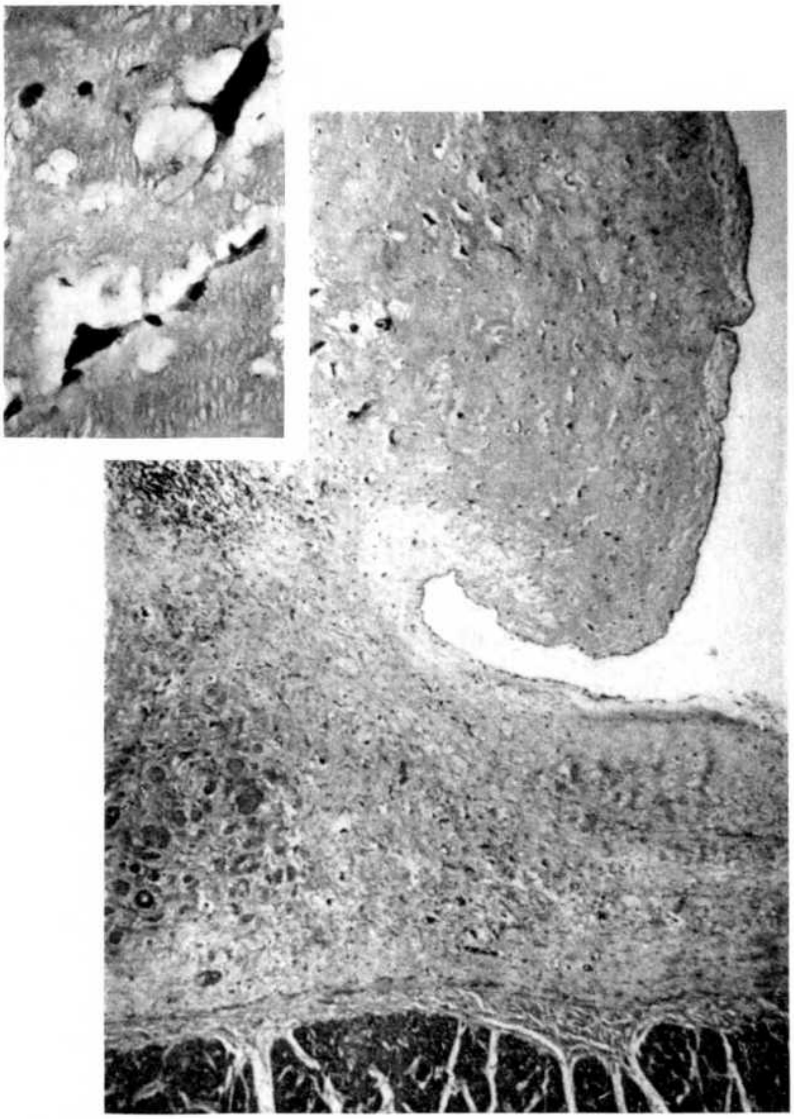

FIGURE 8. Atrial myxoma. A lobular tumour with myxoid stroma containing relatively few angulated cells arises from the myocardium (H & E, × 32). *Inset:* Atrial myxoma. Characteristic multinucleate stellate and kite-shaped cells (H & E, × 320).

certain areas showed prominent myxoid features and was hypocellular. Most areas of the tumours were moderately to densely cellular including spindle-shaped and stellate cells suggestive of fibroblasts (Fig. 6). Mast cells and polymorphonuclear leukocytes were also prominent and these areas of the tumour were fairly richly vascular. No lipoblasts were identifiable in the lesions. The histological appearances suggested the diagnosis of myxoid neurofibroma, particularly in the most recently excised lesion. The histological material was reviewed by Professor D. Mackenzie (Westminster Hospital) who regarded these tumours as most closely resembling myxoid neurofibromata and almost certainly not liposarcomata. Ultrastructural examination of the recent lesion showed scattered Schwann cells (Fig. 7) and mast cells randomly distributed in an otherwise featureless stroma containing aggregates of finely granular material of intermediate electron density and islands of degenerating collagen. The ultrastructural features supported the diagnosis of myxoid neurofibroma.

Atrial tumour. The lesions were typical atrial myxomata made up of a mass of myxoid tissue with randomly distributed characteristic kite-like and stellate cells, some of which were multinucleate (Fig. 8).

DISCUSSION

The combination of atrial myxoma and cutaneous pigmented lesions has been the subject of one previous report (Russell Rees, Ross & Keen, 1973). The patient was similarly a young man with red hair and fair skin, with a striking pattern of cutaneous pigmentation which evidently closely resembled that of our patient. A biopsy of one of the skin lesions in the case described by Russell Rees *et al.* (1973) was reported as showing benign lentigo. We have had the opportunity of reviewing a section from this biopsy and interpret the lesion as an ephelis, not a lentigo. These two patients appear to have the same condition. There was some evidence to support inheritance by an autosomal dominant gene in the case reported by Russell Rees *et al.* (1973). Our case provides little additional information with regard to the pattern of inheritance. Both parents were heavily freckled but in both cases clinical examination, electrocardiogram and echocardiogram were normal.

The principal difference between our case and that reported by Russell Rees *et al.* is the occurrence of subcutaneous tumours in ours. We believe that the precise histological classification of these tumours must remain in question, though they very closely resemble neurofibromata, and this diagnosis is supported by the ultrastructural findings. Although these tumours show gross myxoid degeneration, they do not appear to be true myxomata, and we regard the possibility that these tumours were metastatic atrial myxomata as extremely remote. Such metastases have been described (Ringertz, 1942) but their histological appearance differs considerably from that of our patient's subcutaneous tumours. We have learned that the patient reported by Russell Rees *et al.* (1973) has recently presented at a different hospital with a palatal tumour. This was excised and we have had the opportunity to examine the histological material. The microscopic features are similar to those seen in sections from our own patient's subcutaneous tumours, and the diagnosis of myxoid neurofibroma is even more strongly suggested.

Another combination of cutaneous pigmented lesions (lentigines) and cardiac disease (hypertrophic cardiomyopathy) has been recognized for some time under the name of the progressive cardiomyopathic lentiginosis syndrome; this condition is well reviewed by Polani & Moynahan (1972). The authors suggested that this syndrome represents an anomaly of neural crest elements, though it is unclear to what extent the neural crest contributes to the formation of the heart. A characteristic combination of other features is found in patients with this syndrome, including growth retardation, hypogonadism and sensorineural deafness. These features are conspicuously absent in both our case and that described by Russell Rees *et al.* (1973). Polani & Moynahan (1972) suggested that the cardiomyopathy might represent a response of the heart to circulating metabolites secreted by the melanocytes of the lentigines. They cited the frequent secretory nature of other tumours of neural crest origin such as phaeochromocytomata, ganglioneuromata and malignant melanomata, and suggested as a model the development of fibrous endocardial deposits in the hearts of patients with the carcinoid syndrome. It appears to us highly improbable that atrial myxoma could develop as a response to circulating metabolites derived from cutaneous melanocytes. While the two syndromes are clearly distinct, there are intriguing parallels. The cell of origin of neurofibromata almost certainly derives from the neural crest, and this provides a developmental link with the melanocytes in the pigmented lesions. The characteristic cells of atrial myxomata are, however, believed to be of mesenchymal origin, though the evidence for this is not strong. An alternative view is that they arise from the cardiac endothelium. The combination of atrial myxoma with profuse melanocytic proliferation and neurofibromata suggests the possibility that this tumour too derives from neural crest tissue.

In our view this condition cannot be considered merely as a variant of neurofibromatosis, since none of the pigmented macules are of true 'café-au-lait' type, and there is a conspicuous absence of lesions in the axillae.

In conclusion, this report describes a second case of atrial myxoma associated with cutaneous pigmented lesions. These cutaneous lesions were of various histological types but were predominantly ephelides. A further feature is added in the form of subcutaneous myxoid fibromata which are almost certainly neurofibromata. For those who would like a name for this condition, may we suggest the NAME syndrome; N for naevi; A for atrial myxoma; M for myxoid neurofibromata; E for ephelides. Recognition of the patient with this distinctive syndrome is of the greatest importance, since the potentially disastrous consequences of systemic embolization of atrial tumour fragments can be averted by early operation. Indeed, the patient reported by Russell Rees *et al.* (1973) presented with hemiparesis. Echocardiography is a rapid and non-invasive technique which reliably identifies such tumours, and we suggest that this investigation is indicated in any patient presenting with abnormally profuse macular pigmentation.

ACKNOWLEDGMENTS

We are grateful to Mr H.H. Nixon for referring this patient and to Professor D.H. MacKenzie for reviewing the histology of the subcutaneous tumours. We are also indebted to Dr R.J. Sandry and Dr T.J. Ryan for enabling us to examine histological material from the skin and palate respectively of the case reported by Russell Rees *et al.* (1973).

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Hypertrichosis lanuginosa acquisita

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SUMMARY

The purpose of this report is to give further details of a case of hypertrichosis lanuginosa acquisita, previously documented as not having an associated malignancy, which at post-mortem was found to have evidence of widespread internal malignant disease.

There have been reports in the literature of more than twenty cases of hypertrichosis lanuginosa acquisita, a condition characterized by an abnormal growth of fine hair similar to the lanugo hair normally present during prenatal life. Such excessive hair growth, referred to in some cases as vellus-like, usually commences suddenly on the face, involving the cheeks, chin, upper lip, nose, forehead and ears. There is often progressive involvement of the neck, shoulders, limbs and trunk, but the palms and soles, which have no hair follicles, are spared. Excessive terminal hair growth has been reported on occasions. Fretzin (1967) introduced the term 'malignant down' owing to the association of several cases with malignant disease, and since then all further reports have had associated malignancy. The following patient, previously shown at the Endocrinology Section of the Royal Society of Medicine (Le Marquand & Bohn, 1951) as an unusual case of recurrent gastro-intestinal ulceration and acquired lanugo hair growth but without associated malignancy, was found at post-mortem to have a primary carcinoma of the lung and widespread metastases.

CASE REPORT

A 61-year-old man, after 28 years of male pattern baldness, developed a thick golden brown growth of hair on the scalp quite different from the original which was light brown. On the face, forehead, eyelids and ears there was a downy lanugo type of hair and on the rest of the body it was transitional between lanugo and ordinary. This abnormal hair growth commenced after the second of three operations for severe gastro-intestinal ulceration.

One month after a prostatectomy the patient came to hospital as an emergency haematemesis. A large duodenal ulcer was dealt with by partial gastrectomy. At his second emergency admission 6 months later he was found to have a huge gastro-jejunal ulcer, and on this occasion an almost complete gastrectomy was performed, leaving behind a small cuff of stomach at the cardia. In spite of this he developed further jejunal ulceration 3 weeks later requiring a total gastrectomy (between these

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two operations the abnormal hair growth commenced). Histology of the stomach showed a very large number of oxyntic cells but at no stage was there any evidence of malignancy and his chest X-ray was normal.

Three months later the patient was noted to have black pigmented areas on the dorsum of the tongue and on the buccal mucosa. He began to experience increasing salivation, waterbrash and heartburn, having earlier complained of some disturbance of taste. He became extremely weak, lost weight, and had a persistently low blood pressure. Although biochemical confirmation of suspected adrenal impairment was not obtained there was an initial improvement with deoxycortone (a mineralocorticoid) and methyl testosterone treatment. The abnormal hair growth continued, and a number of discrete raised pigmented areas of skin were seen on the trunk and neck. Sixteen months after the first appearance of the lanugo hair his general condition deteriorated and he developed a productive cough and clubbing of the fingers and toes. A chest X-ray showed right hilar shadowing suggestive of neoplastic disease. He died a few months later and at post-mortem was found to have an infiltrating tumour of the right main bronchus (type—solid polygonal-celled, in places columnar, mucus secreting and tubular carcinoma). There were metastatic deposits in the liver, thyroid, lungs and adrenal glands. Although both adrenal glands were greatly enlarged there was a substantial amount of normal residual cortex and medulla. The pituitary was normal and it was concluded that there was no pathological evidence to support the diagnosis of Addison's disease. The pancreas was also normal.

DISCUSSION

This patient has hitherto frequently been quoted as an example of hypertrichosis lanuginosa acquisita not associated with internal malignancy. This is not so, and our purpose in reporting this case is to put the record straight. At the time of publication in 1951 the significance of the unusually severe recurrent gastro-intestinal ulceration was not known. Such a history today would lead to a confident diagnosis of a gastrin-secreting tumour somewhere, but Zollinger & Ellison's paper was not published until 1955 and gastrin studies were not available then. The source of gastrin production is almost always the pancreas. There is some similarity between our case and that reported by McLean & Macaulay (1977) of the 19-year-old girl who had hypertrichosis launuginosa acquisita and massive duodenal ulceration. She had markedly elevated serum gastrin levels, and died 8 months after the onset of symptoms from a metastasising pancreatic islet cell carcinoma. It is possible that ectopic production of gastrin, or a substance with gastrin-like properties, from an early carcinoma of the lung was responsible for the severe ulceration in our patient.

The occurrence of pigmentation of the mouth has also been recorded in two other cases of hypertrichosis lanuginosa acquisita, both with associated carcinoma of the lung (Fretzin, 1967; Ikeya, Izumi & Suzuki, 1978). Other features relating to the mouth have been reported, such as pain or soreness of the tongue and buccal mucosa, disturbance of taste and changes in the appearance of the tongue, such as 'furrowing', redness, and the occurrence of papules.

There now remain only three cases of hypertrichosis lanuginosa acquisita with no documentation of malignant disease, and in all three investigation and follow-up were not adequate. The case of acanthosis nigricans and hypertrichosis described by Morris (1894) died soon afterwards of anuria, but no post-mortem was permitted. In the case described by Le Double & Houssay (1912) the disorder appeared following 'influenza' and in the case reported by Ormsby (1930) there was evidence of a raised metabolic rate. All other recorded cases have had underlying malignancy and therefore there is now no fully documented case of this condition not associated with malignant disease to be found in the current literature. The condition must be distinguished from acquired hypertrichosis induced by various drugs and following severe shock or brain injury, but this type is reversible and easily diagnosed from the history.

The source of the primary growth in other reports has been the colon (Hegedus & Schorr, 1972, (two cases); Van der Lugt & Dudok de Wit, 1973; Davies *et al.*, 1978), rectum (Chadfield & Khan 1970; Djajadiningrat *et al.*, 1970), gall-bladder (Herzberg, Potjan & Gebauer, 1968), pancreas (McLean & Macaulay, 1977), urinary bladder (Lyell & Whittle, 1951), lymphoma (Samson *et al.*, 1975), uterus (Samson *et al.*, 1975; Kaiser, Perry & Yoonessi, 1976), ovary (Dingley & Marten, 1957), breast (Turner, 1865; Wadskov, Bro-Jørgensen & Søndergaard, 1976) and lung (Fretzin, 1967; Hensley & Glynn, 1969; Rzempoluch, Szczurek & Osiecki, 1976; Ikeya *et al.*, 1978). It can now be stated that the sudden onset of generalized excessive growth of lanugo-like or terminal hair is a cutaneous manifestation of internal malignancy which may antedate the diagnosis of neoplasia by at least a year. We support the suggestion previously made in other papers, that this may well be the result of the secretion, by primitive tumour cells, of some atavistic or vicarious hair-producing hormone not yet identified.

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Porokeratotic eccrine ostial and dermal duct naevus

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SUMMARY

With rare exceptions, the presence of cornoid lamellae in skin biopsy specimens is considered diagnostic of porokeratosis. Since the initial descriptions of this condition by Mibelli (1893) and Respighi (1893), there has been debate concerning its relationship to the eccrine sweat duct.

This paper describes an epidermal naevus, which pathologically demonstrated gross examples of cornoid lamellae associated exclusively with the eccrine duct and ostia, and which appears to represent a naevus or benign hamartoma of these structures. This entity needs to be clearly differentiated from porokeratosis of Mibelli.

The term 'porokeratosis' was first used by Mibelli in 1893 in his classic description of the condition which now bears his name. It indicated his belief that, pathologically, the ring of abnormal keratinization, characteristic of this lesion, originated in and developed from the sweat gland pore. In the same year, Respighi, describing similar lesions, questioned this interpretation, and since then only rarely have publications appeared attempting to confirm Mibelli's original concept (Saunders, 1961; Sehgal & Dube, 1968). Involvement of the sweat duct and follicular orifices is now considered fortuitous, being but a transient phase of the migratory process of altered clones of epidermal cells (Pinkus, 1961; Reed & Leone, 1970). Currently, porokeratosis is used as a clinical diagnostic title for a group of epidermal disorders, which are considered to evolve along a similar pathophysiological pathway. These disorders begin as tiny papules, which by gradual peripheral extension produce variably sized plaques, with raised annular margins. The pathological counterpart of the raised annulus is a sharply defined column of parakeratotic horn arising obliquely from a thinned, often invaginated epidermal base, devoid of a granular layer—'the cornoid lamella'.

In 1967, Chernosky & Freeman documented essentially similar but more diminutive lesions arising on sun exposed skin, so that porokeratosis may now be classified into four major groups: plaque (Mibelli), superficial disseminated, linear or zosteriform, and disseminated superficial actinic types (Mikhail & Wertheimer, 1968; Lever & Schaumburg-Lever, 1968).

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Porokeratotic pathology has also been described as occurring in cases of punctuate keratoderma of palms and soles (Brown, 1971; Herman, 1973), and more recently as an isolated finding in a case of pachyonychia congenita (Wilkin, Rosenberg & Kanzaki, 1978). These rare conditions excepted, the presence of a cornoid lamella in biopsy tissue is considered diagnostic of porokeratosis. This paper describes yet another exception.

CASE REPORT

A 3-year-old Caucasian girl was brought to the clinic by her mother because of a symptomless lesion of her left foot which had been present from birth. After a normal gestation and delivery, a warty lesion was noticed along the distal third of the inner side of the left foot extending to the tip of the great toe. Gradual proximal extension had occurred by the separate appearance of new keratoses.

Examination revealed a linear keratotic lesion, consisting of tiny discrete papules (Fig. 1) which extended along the medial border of the left foot, two thirds the distance from the great toe to the malleolus.

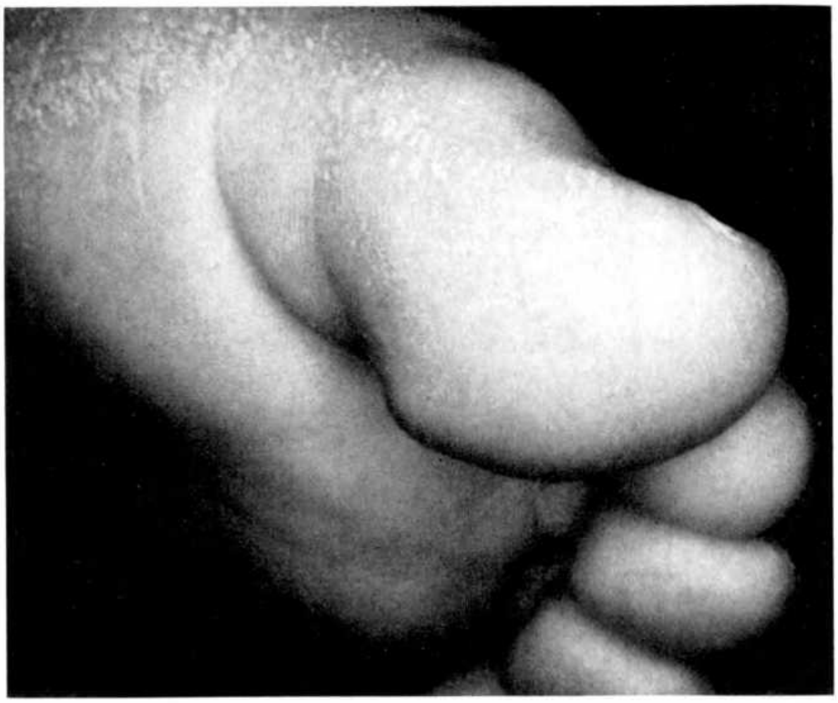


FIGURE 1. Keratotic papules in linear distribution (left foot).

Both feet were symmetrical. No other cutaneous or skeletal abnormality was present, and general physical examination was normal. Starch-iodine preparations, after heat-induced sweating, demonstrated focal anhidrosis corresponding exactly to the warty lesions.

A family history revealed two normal siblings, a 7-year-old paternal cousin with an extensive giant congenital naevus, and another 5-year-old paternal cousin said to be an hermaphrodite.

Histopathology

A 4 mm punch biopsy was obtained under local anaesthetic from the medial aspect of the left foot close to the plantar surface, fixed in Zenker's fluid and routinely processed. Serial haematoxylin and eosin sections were examined throughout the tissue block and the following histochemical stains performed: PAS with and without diastase digestion, Masson's trichrome, Verhoff van Gieson's, Fontana (melanin) and Feulgen reaction.

The specimen showed hyperkeratosis and acanthosis characteristic of plantar skin. Pilosebaceous units, apocrine glands and erector pili muscle were absent. Large numbers of cornoid lamellae were seen in all the tissue sections. The majority arose at the level of the mid dermis from within epidermal invaginations (Fig. 2) and others from more superficial levels (Fig. 3). In each case, either directly or by serial section, these deep epidermal invaginations could be shown to be grossly dilated eccrine ducts. Those lamellae arising superficially were also demonstrated, without exception, to be related to sweat duct structures but were of two distinct appearances. Most of them were essentially similar to the deeper lesions but were confined to the epidermal portion of the duct. A few lesions arose immediately surrounding the acrosyringium, sleeving the 'horny cork screw' (Fig. 3). Occasional entirely normal eccrine ducts were present (Fig. 4).

Serial sections clearly demonstrated the focality of each individual lamella, none being seen to pass beyond the eccrine unit. Those sleeving the acrosyringium could not be shown to be part of a more extensive ring structure.

In addition, abnormalities of the dermal ducts extended below the origin of the cornoid lamella but the coiled glandular portion appeared normal throughout. These abnormalities consisted of gross benign hyperplasia of the lower part of the duct and patent, dilated lumen (Fig. 5).

All cornoid lamellae conformed to the classical pattern, showing focal compact parakeratotic horny columns arising from pale staining squamous epithelium showing variable but often pronounced vacuolation (Fig. 4). The granular layer was deficient but no dyskeratotic cells were seen. The dermis and epidermis were otherwise normal; specifically no evidence of connective tissue atrophy, anomaly, or of an inflammatory infiltrate was detected. The parakeratotic material of the lamellae was Feulgen positive indicating presence of DNA. Glycogen was demonstrated in the epidermal cells surrounding the base of the cornoid lamellae and in the clear cells of the eccrine glands.

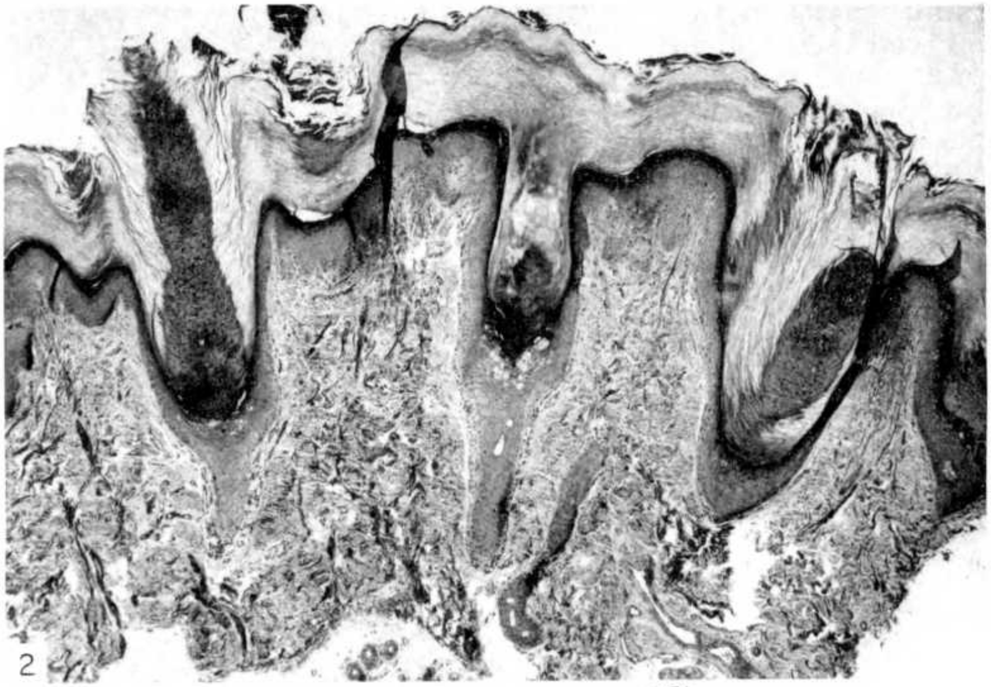
DISCUSSION

The clinical presentation and development of this case suggested a linear epidermal naevus. Indeed, biopsy might not have been undertaken had it not been for the mother's extreme concern that her child might have a potentially malignant defect.

Pathologically, the lesion showed distinctly focal cornoid lamellae in large numbers. Closer examination demonstrated that the abnormality was confined to eccrine ducts, that involvement was variable, but that the whole length of the duct could be affected. The glandular portion of the unit was uninvolved throughout.

Sweat gland function could not be assessed adequately, but the lesion was anhidrotic presumably due to ductal occlusion.

That the lesion was present at birth is also uncharacteristic of porokeratosis, typically an acquired defect. The growth of the abnormality is consistent with either an epidermal naevus or a linear porokeratosis, but there are no annular lesions to support the latter diagnosis. It might be argued that the lesion represents a linear form of porokeratosis in which there has been a failure or delay in migration, but the pathological changes are more suggestive of a localized and stable defect of the eccrine duct.



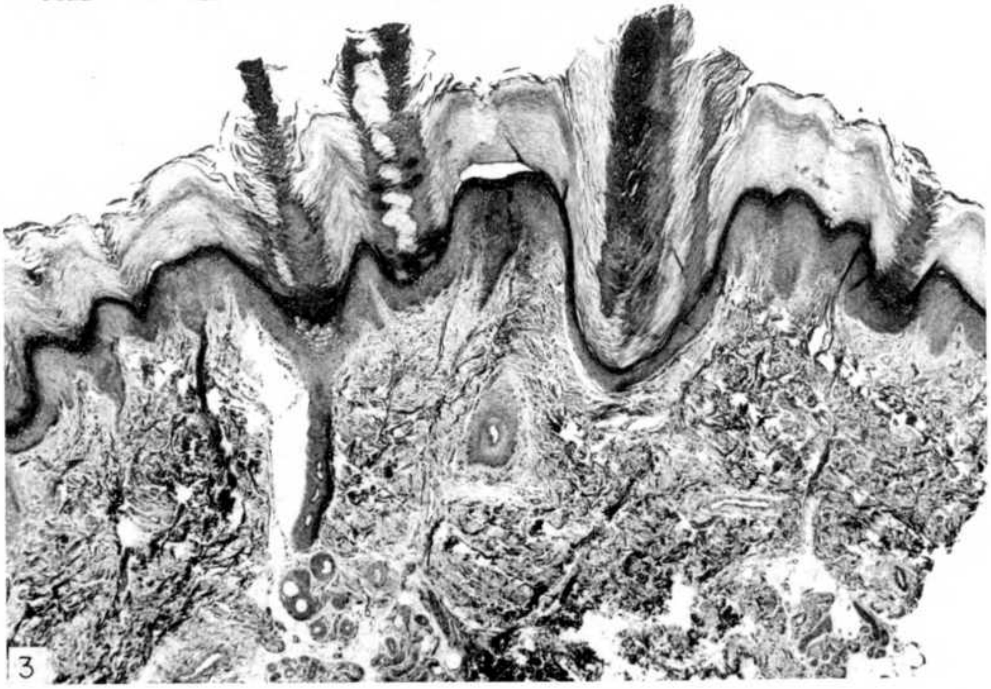


FIGURE 2. Cornoid lamellae arising from deep epidermal invaginations (haematoxylin-eosin, × 32).
FIGURE 3. Cornoid lamellae arising from acrosyringium and sleeving acrosyringium (haematoxylin-eosin × 28).

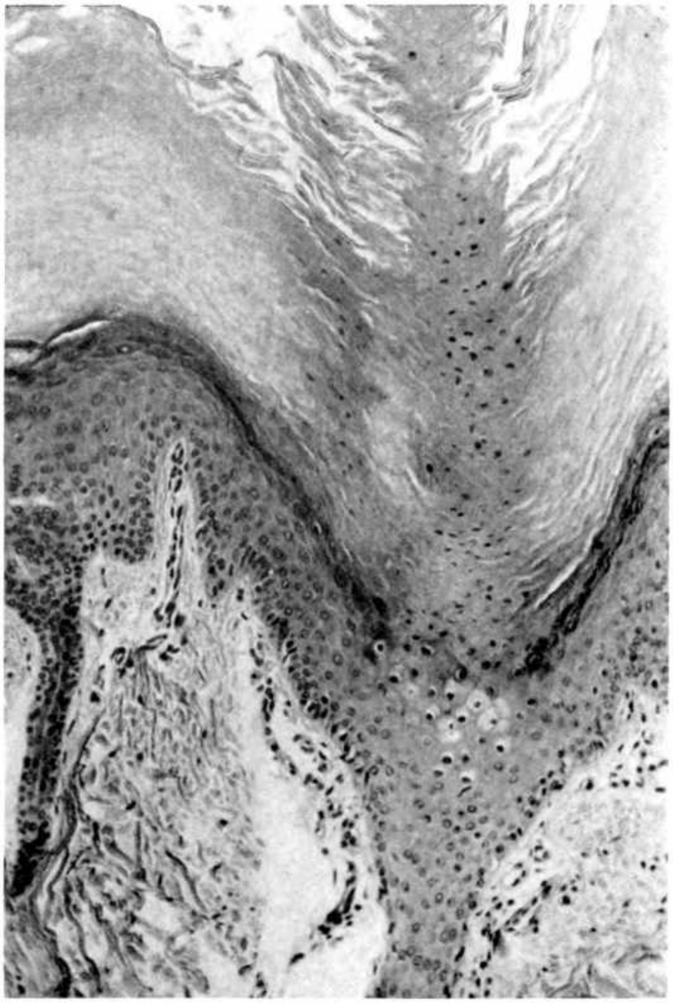


FIGURE 4. Acrosyringial cornoid lamella, with adjacent normal eccrine duct (haematoxylin-eosin, × 100).

Despite this child's lesion having the clinical appearance of an epidermal naevus, this diagnosis is readily excluded histologically. In 1977, Dupre & Christol described a small number of cases of inflammatory linear verrucose epidermal naevus in which the hyperplastic epidermis demonstrated alternating and sharply defined segments of ortho and parakeratotic horn. They suggested that this pathological picture might occasionally resemble porokeratosis. Closer examination shows only a very superficial similarity, however, as the Malpighian layer has none of the characteristic changes of porokeratosis.

We have been unable to find any similar case in the literature. In the absence of other detectable dermal abnormality it is impractical to classify this lesion as an organoid naevus. It is also quite clearly different from previously described eccrine naevi. The uncommon naevus sudoriferous (Goldstein, 1967) presents usually as an acquired area of hyperhidrosis and pathologically shows an excess of mature functioning eccrine units. A rare eccrine angiomatous hamartoma (Hyman, Harris

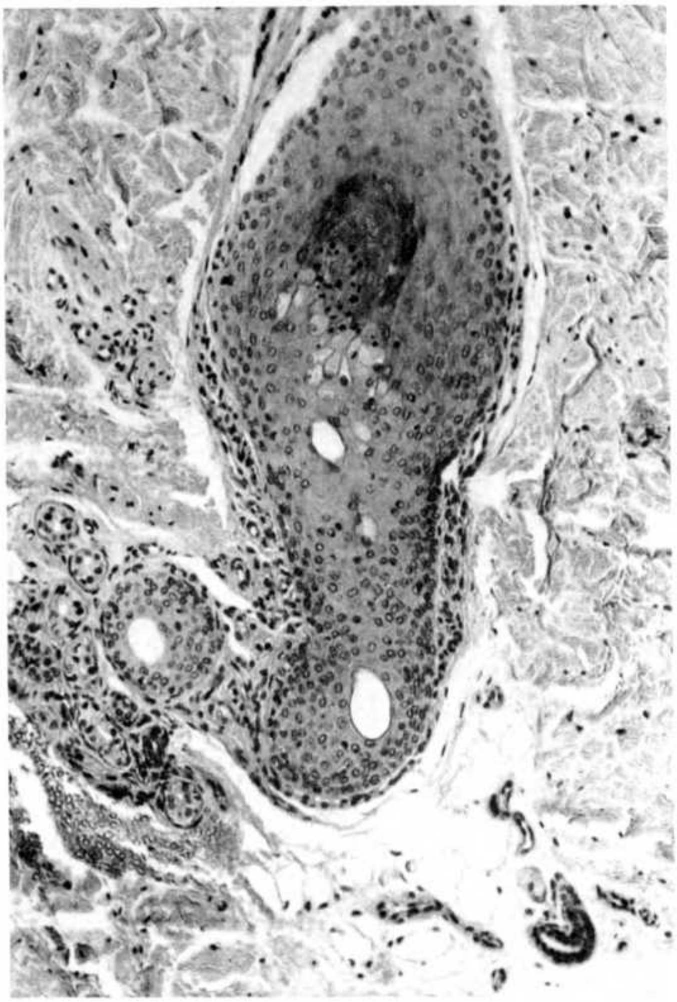


FIGURE 5. Normal eccrine gland in continuity with hyperplastic duct. Note lumen and origin of cornoid lamella (haematoxylin-eosin, × 100).

& Brownstein, 1968) is also described as a focal dermal nodule arising usually in childhood, and often clinically suggestive of a haemangioma. The pathology of this lesion reveals increased numbers of sweat glands set in an angiomatous stroma or malformation. This child's condition is quite different from the punctuate keratodermas showing porokeratosis, both clinically and pathologically (Brown, 1971; Herman, 1973), and indeed both these cases were also acquired.

It would seem most unlikely that this pathological entity is unique, but it would certainly have been missed had the apparently innocuous epidermal naevus not been biopsied.

The current theory of Reed & Leone (1970) suggests that the lesions of porokeratosis, of all types, are produced by the migration of abnormal epidermal clones. Involvement of the appendage structures is a consequence of this migratory process and in its wake a subtle, but occasionally an obvious, degree of atrophy of the epidermis, appendages and papillary dermis remains. This biological activity is distinctly at variance with the stationary hamartomatous lesion we have described.

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Endocervical adenocarcinoma and vulval Paget's disease: a significant association

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SUMMARY

Extra-mammary Paget's disease is an uncommon entity. It has been associated with an underlying sweat gland carcinoma. This paper presents a patient with metastatic endocervical carcinoma and vulval Paget's disease. We believe that the Paget's disease developed as a direct consequence of the cervical tumour. In patients with extra-mammary Paget's disease with no sweat gland carcinoma a careful search to exclude other primary malignant tumours is advised.

CASE REPORT

The patient, a 55-year-old Caucasian, came to the gynaecology clinic complaining of long-standing severe vulval itching. The symptoms began 3 years after her menopause at 47 years. A biopsy had been performed in the U.S.A. but we could not trace the result although she was told that there was no evidence of malignancy.

On examination the labia minora were erythematous and showed several white patches. The clitoris, labia minora and majora were inflamed and tender. On vaginal examination there was a hard mass extending from the cervix into the right parametrium. Biopsies were taken from the cervix and vulva. X-rays and a liver scan showed multiple skeletal and hepatic metastases with obstruction of the right ureter.

The patient developed bronchopneumonia and she died 2 weeks after admission. Permission for an autopsy was obtained.

PATHOLOGY RESULTS

Vulval and cervical biopsies

The vulval and cervical specimens were fixed in 10% formaldehyde and processed by standard techniques. Sections (5 μm) were stained with haematoxylin and eosin, PAS with and without diastase, alcian blue at pH 2·5, mucicarmine, toluidine blue and Masson-Fontana.

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Vulva

There was patchy hyperkeratosis and parakeratosis with areas of ulceration. The epidermis was hyperplastic and showed the changes of Paget's disease especially prominent in the lower layers. The infiltrating cells were seen as clusters throughout, and were large and pleomorphic with irregular, hyperchromatic nuclei (Fig. 1). Cytoplasm varied from scanty to abundant and was consistently clear staining. Scattered mitoses were observed. Intercellular bridges and keratinization were not present. The PAS with and without diastase, and alcian blue stains were positive. The toluidine blue showed marked metachromasia. The muci-carmine was negative and Masson-Fontana showed occasional melanin granules. The dermis contained scattered malignant cells. Sweat gland ducts were free from tumour and an underlying primary carcinoma was not identified.

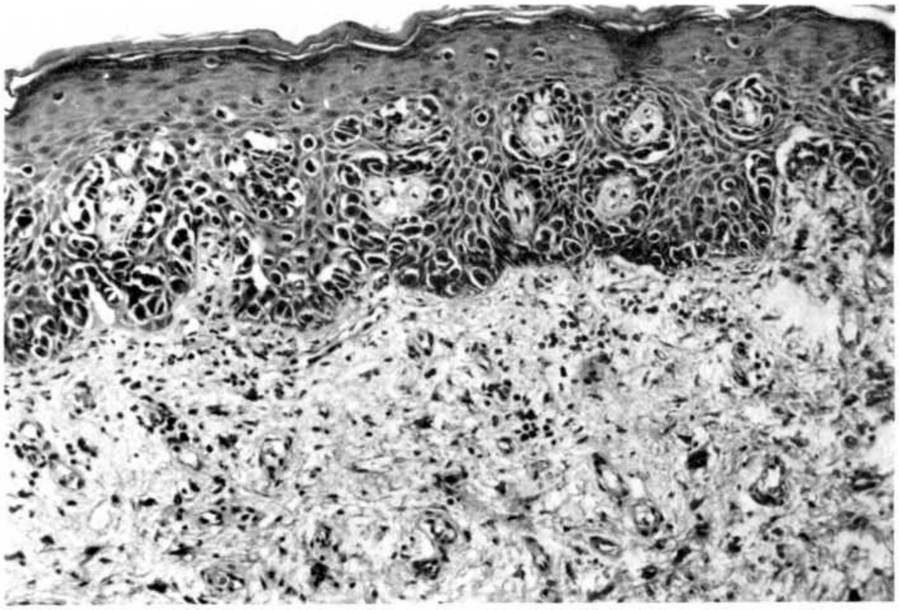

FIGURE 1. Vulval biopsy (H & E, × 48).

Cervix

The squamous epithelium was normal. The underlying tissue was infiltrated by a carcinoma with malignant cells in varying arrangements ranging from single isolated cells to an 'Indian-file' arrangement with occasional clusters and some tendency in places to glandular formation (Fig. 2). The individual cells were quite pleomorphic and had irregular hyperchromatic nuclei. The PAS with diastase was positive in occasional cells; toluidine blue showed marked metachromasia. Alcian blue was weakly positive. Muci-carmine and Masson-Fontana were negative. The features were considered to represent a rather poorly differentiated endocervical carcinoma.

Autopsy

The vulva showed features as described above. Situated in the cervix and extending into the right vaginal fornix was a 3 × 2 × 1 cm hard, white tumour mass. Metastases were found in the myometrium, internal iliac and paratracheal lymph nodes. There was a severe bilateral bronchopneumonia. The remainder of the autopsy including examination of the breasts was normal.

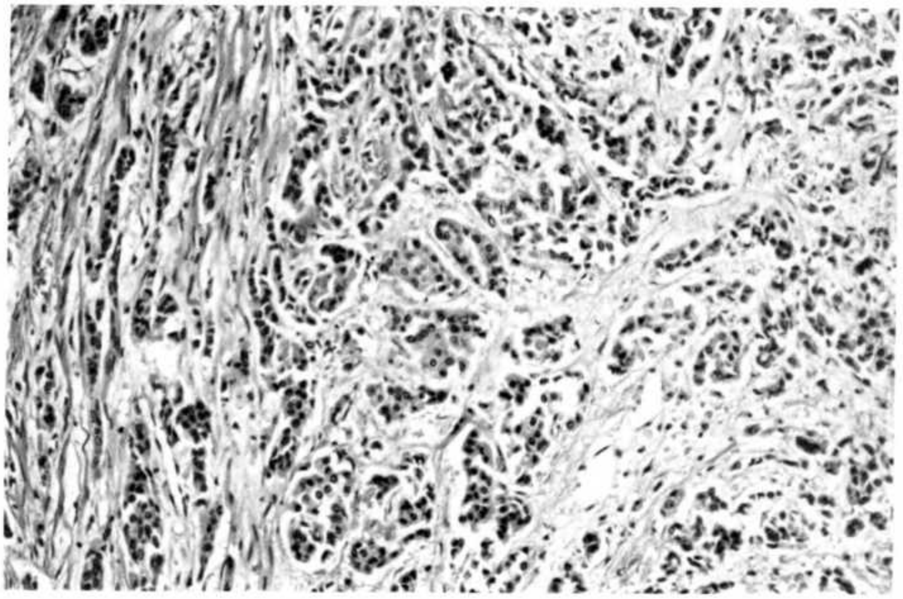

FIGURE 2. Cervix. In this area the tumour shows a tendency to gland formation (H & E, × 100).

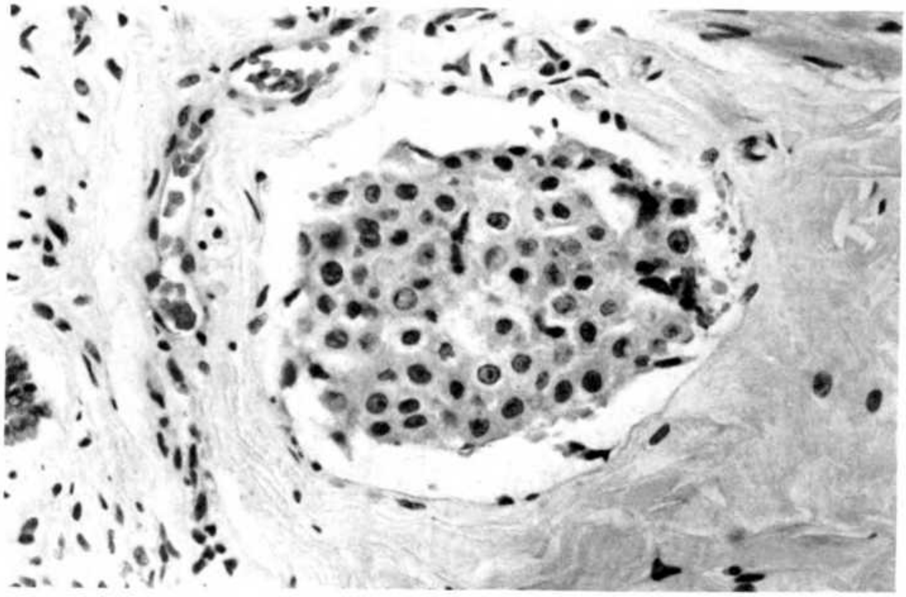

FIGURE 3. Vulva showing lymphatic spread (H & E, × 450).

The vulva was removed and made into eighty-eight blocks. Sections were examined and features similar to those described above were observed. However, in places there was a marked tendency to involve hair follicles and superficial portions of sweat ducts. In several sections tumour-filled lymphatics were seen (Fig. 3). A sweat gland carcinoma was not identified.

The cervical tumour showed features similar to those described for the biopsy and the metastases were generally of de-differentiated carcinoma although in places the glandular pattern was more prominent and mucus secretion evident.

DISCUSSION

Paget's disease of the breast (Paget, 1874) is well recognized as a specific entity of the nipple and areola associated with an underlying mammary cancer of ductal origin (Ashley, 1978). Less readily understood is extra-mammary Paget's disease. Shortly after Paget's initial description scrotal (Cocker, 1889) and vulval (Dubreuilh, 1901) involvement were recognized. Since then a variety of sites have been recorded including axilla and umbilicus (Koss, Ladinsky & Brockunier, 1968; Ueki & Kohda, 1979).

Just as mammary Paget's disease has been shown to be associated with a duct carcinoma, vulval Paget's disease has been associated with carcinoma of sweat gland origin (Mitchell, 1974; Roth, Lee & Ehrlich, 1977; Breen, Smith & Gregori, 1978). Other reports record extra-mammary Paget's disease in association with other primary growths including prostatic carcinoma (Merino, Livolsi & Lytton, 1978) and rectal cancer (Jackson, 1975; Williams, Rogers & Quan, 1976). There also appears to be a significant association of anogenital Paget's disease with neoplasia elsewhere in the body. Thus Helwig & Graham (1963) showed that 25% of their forty patients had malignancies elsewhere in the body (excluding sweat gland carcinomas). Lee *et al.* (1977) in a series of thirteen patients described four with internal malignancy including squamous carcinoma of cervix, transitional carcinoma of bladder and basal cell carcinoma of foot. A fourth patient died of carcinomatosis of unknown origin. Fenn, Morley & Abell (1971) presented an interesting patient with vulval Paget's disease who had no less than four separate malignancies including carcinoma of breast, basal cell carcinoma of skin, malignant melanoma and an ileal carcinoid tumour.

Paget's disease has also been described at distant sites in association with carcinoma of ceruminous glands (Fligiel & Kaneko, 1975; Michell, personal communication) and of meibomian glands (Boniuk & Zimmerman, 1968).

Mammary Paget's disease is now generally accepted as resulting from an upward migration of tumour cells from the duct into the overlying squamous epithelium. Just exactly why this occurs is unknown and many workers express this phenomenon as epidermotrophism. The histogenesis of extra-mammary Paget's disease is more problematical. A variety of theories have been proposed. Thus Lee *et al.* (1977) believed that the Paget's cells migrate into the epidermis, via the sweat duct, from an underlying sweat gland carcinoma. Helwig & Graham (1963) believed that Paget's disease has a multicentric and autochthonous origin and thus can involve the epidermis alone, the epidermis and underlying sweat gland or the epidermis and adjacent internal organs. Pinkus & Mehregan (1963) suggested that Paget's cells might be derived from the poral portion of the sweat duct. This could certainly be an explanation for those instances of Paget's disease where there was involvement of the epidermis alone. Greenwood & Minkowitz (1971) described a case of mammary Paget's disease associated with a breast duct carcinoma. A subsequent metastasis to the ipsilateral arm had Paget's disease of the overlying epidermis with several areas where the dermal tumour seemed to be directly infiltrating the epidermis. Although there are alternative postulates it is possible that in this case the duct carcinoma had epidermotrophic properties which resulted in Paget's disease of the arm.

The association of the endocervical mucus secreting carcinoma and Paget's disease has to our knowledge only been reported once previously (Jones, Austin & Ackerman, 1979).

In this instance the histochemical uniformity of the vulval and cervical lesions, the presence of tumour in the dermis and also within vulval lymphatics suggested to us that the Paget's disease represented a metastatic manifestation of the cervical cancer possibly on the basis of retrograde lymphatic spread from the perivaginal lymphatics to the vulva.

We believe that in patients with extra-mammary Paget's disease with no sweat gland tumour a careful search to exclude other primary malignant tumours is advised. Indeed, recently we have seen a case of peri-anal Paget's disease in a man of 62 years who proved to have an underlying primary prostatic carcinoma.

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Sweet's syndrome in infancy

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SUMMARY

A 3-month-old Japanese girl developed tender erythematous elevated plaques on her face and buttocks with fever up to 38°C. Histology showed a perivascular neutrophil infiltrate with nuclear debris in the dermis. Endothelial cells were swollen but no hyalinization of the vessel wall was observed. The lesions recurred several times and responded little to antibiotics but dramatically to oral corticosteroid. We diagnosed her skin condition as Sweet's syndrome in infancy.

CASE REPORT

A 3-month-old Japanese girl developed tender, dark erythematous, raised plaques on her face and buttocks in November, 1976, 1–2 days after flu-like symptoms of fever as high as 38°C and rhinorrhoea. The lesions persisted for 2 weeks and subsided after commencing treatment with oral antibiotics and a topical steroid. One month later, she was admitted to our hospital because of recurrence of her skin lesions. On physical examination, there were dark erythematous elevated plaques up to 10 cm in diameter on her forehead, cheeks, buttocks and left thigh (Fig. 1). They were tender on pressure. The lesions on the buttocks showed several pea-sized vesicles at their centres.

Abnormal laboratory findings were as follows; W.B.C. 16,100/mm^3 with 66% neutrophils, E.S.R. 60 mm/h, serum IgG 1220 mg/dl, IgA 230 mg/dl, IgM 200 mg/dl.

Histopathology of a skin lesion revealed an acanthotic epidermis and perivascular infiltration of neutrophils in the dermis (Fig. 2). Nuclear debris was scattered in the perivascular infiltrate. The endothelial cells in the upper and mid dermis were swollen; however, hyaline degeneration of the blood vessel wall was not detected. She showed a positive intradermal test to candida antigen and then developed an elevated erythematous plaque at this site 10 days later. Histopathology of the plaque showed exactly the same finding as in her skin lesion. A week after admission to hospital the skin lesions began to subside gradually without any treatment. She was discharged 1 month later. During the follow-up period of 3 years the lesions recurred several times and extended to her trunk. Leukocytosis and fever were not observed with these later recurrences. Oral antibiotics such as cephalosporin and minocycline were given for the recurrences with only temporary effects. For her latest recurrence she was given prednisolone 10 mg/day, as the lesions extended to her face, trunk and extremities. The lesions responded dramatically to the oral steroid therapy and cleared within a week. After gradual withdrawal of oral steroid she has been clear for 6 months.

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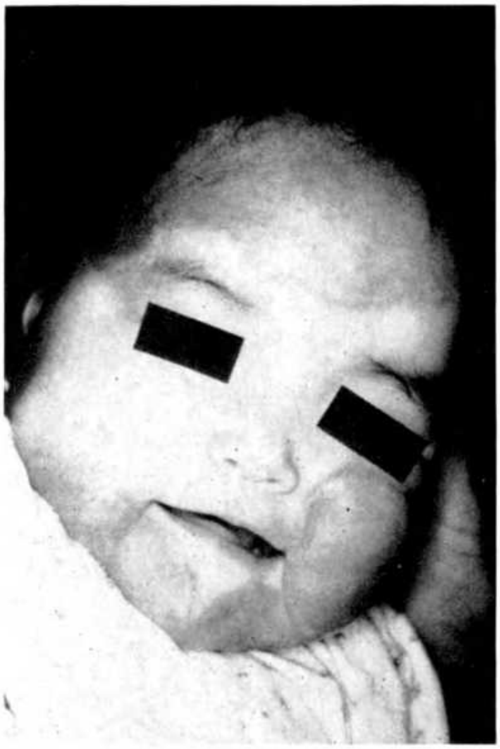

FIGURE 1. Raised erythematous plaques on the face.

COMMENT

Since Sweet described a new entity of tender erythematous plaques in 1964, more than 100 cases have been reported from all over the world. Most of the patients were women aged between 30 and 60 years (Shapiro, Chorhes & Leonard, 1971). The youngest case reported was a 16-year-old boy who was diagnosed as having Whittle's disease, which is a variant of acute febrile neutrophilic dermatosis of Sweet (Finn & Main, 1956).

The new entity includes the following characteristics: raised red plaques, pain or tenderness, fever, polymorphonuclear leukocytosis, characteristic histological picture and good response to corticosteroids (Sweet, 1964).

Although our case was young compared to the cases reported as Sweet's syndrome, she showed recurrent tender raised erythematous plaques with fever up to 38°C and leukocytosis. Histology showed heavy infiltration of polymorphonuclear leukocytes and nuclear debris around blood vessels in the upper and mid dermis. These findings are those of Sweet's syndrome (Sweet, 1964) except the swollen endothelial cells in the dermis. Sweet showed non-vascular involvement electon microscopically (Sweet, 1979). However, it becomes acceptable if the Arthus reaction is accepted as the pathogenesis of Sweet's syndrome, because swollen endothelial cells are a common feature in the Arthus reaction (Roitt, 1977). Therefore, our case would be the youngest of Sweet's syndrome ever reported.

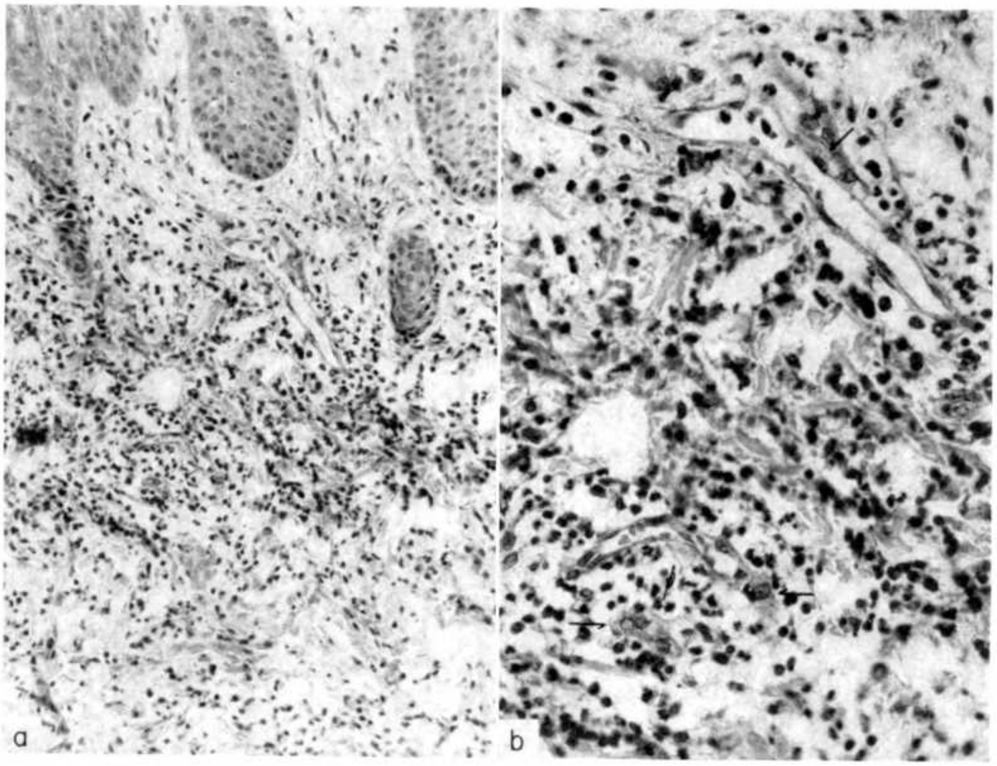

FIGURE 2. Histology of the lesion. (a) Polymorphonuclear leukocyte infiltration in the upper dermis. (b) Nuclear debris, erythrocyte extravasation and swollen endothelial cells (arrow) were noted.

She showed a positive skin reaction to candida antigen and then developed a tender raised erythematous plaque at the tested site 10 days later. This lesion showed a histological reaction similar to that of her involved skin. Although we were not able to amplify on the relationship between candida antigen and her lesion, candida antigen could be one of the aetiological factors for her skin lesions.

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Neonatal acne vulgaris: a possible feature of the fetal hydantoin syndrome

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SUMMARY

A baby with the fetal hydantoin syndrome was found to have acne vulgaris of the face. Hydantoin taken by epileptic women during pregnancy may cause neonatal acne in the offspring.

According to Hoekelman *et al.* (1978) about 10% of children whose mothers receive hydantoin during pregnancy are born with the fetal hydantoin syndrome. The main features of this syndrome are mental and growth retardation, unusual facies, digital and nail hypoplasia and coarse scalp hair. Authoritative reports (Speidel & Meadow 1972; Hill *et al.* 1974) make no mention of the presence of acne vulgaris.

The baby to be described was found to have facial acne vulgaris in addition to the fetal hydantoin syndrome.

CASE HISTORY

A woman of 31 years had been treated with hydantoin and phenobarbitone since 1971 for grand mal seizures and was on the same treatment throughout the whole of her two pregnancies. In June 1979 she gave birth to a full term male baby with a congenital heart lesion, absence of the nails of both little fingers and luxuriant scalp hair; features of the fetal hydantoin syndrome. At 3 days of age acneiform lesions appeared on the chin (Fig. 1) and have been present over 3 months. No oils or creams were applied to the baby's face. Neither of the parents suffer from acne vulgaris. An older brother, born in 1977, was reported to have suffered from a similar facial eruption but he did not show the typical features of the fetal hydantoin syndrome.

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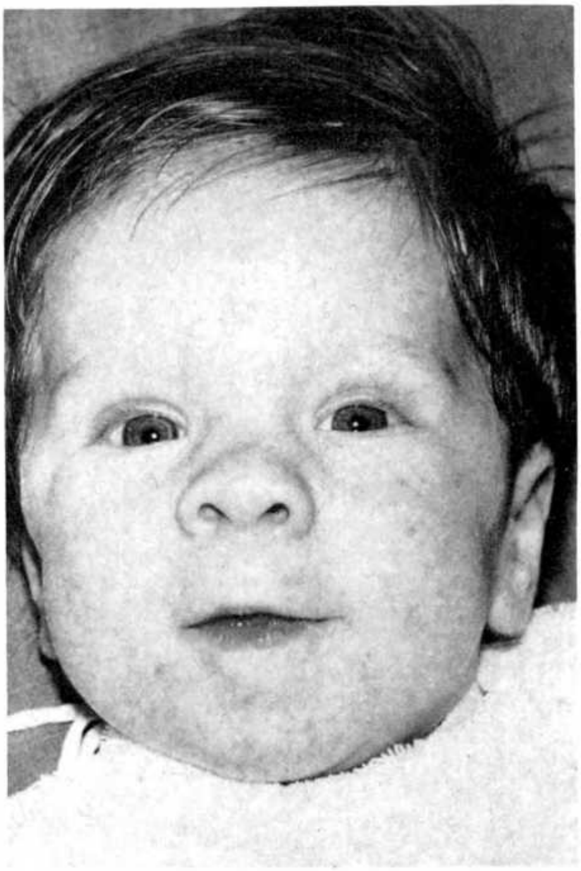

FIGURE 1. Acneiform lesions on the chin of patient.

DISCUSSION

Neonatal acne vulgaris may present at 2 weeks as a mild self-limiting dermatosis or more commonly it appears after several months in a more severe and protracted form (Plewig & Kligman, 1975).

Infantile acne vulgaris is a well-recognized entity, the cause of which is not known (Cunliffe & Cotterill, 1975). Hereditary influences (Bessone, 1958) as well as excessive androgenic activity associated with sexual precocity and virilism are contributory factors, as is contact with certain oils (Berlin, 1954). Treatment of pregnant women with systemic halogens and corticosteroids may result in neonatal acne vulgaris (Cunliffe & Cotterill, 1975).

Acne vulgaris can be provoked in epileptic subjects who are treated with phenobarbitone but the precise mechanism is not fully understood (Rook, Wilkinson & Ebling, 1972). Although phenobarbitone crosses the placenta (Moya & Thorndike, 1962) there is no mention of acne vulgaris in babies born to mothers taking this drug (Desmond *et al.*, 1972).

Hydantoin is reported to cause numerous skin reactions including hypertrichosis of the extensor surfaces of the limbs (Levantine & Almeyda 1972). Jenkins & Ratner (1972) reported worsening of existing acne which improved when hydantoin treatment was stopped. In pregnant women hydantoin crosses the placenta (Mirkin, 1971).

In the patient described, neonatal acne could have resulted from treatment of the pregnant mother with phenobarbitone but its association with the fetal hydantoin syndrome suggests that hydantoin may have been responsible. The presence of acne vulgaris in the newborn should alert the physician to other features of the fetal hydantoin syndrome.

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Comment

ON THE AETIOLOGY OF PSORIASIS

Many different theories regarding the cause of psoriasis have been proposed (Mier & Cotton, 1976). Most of these ideas are now frankly obsolete. Some lie dormant, liable to be awakened by chance observation. A few—such as the concept of Voorhees & Duell (1971)—remain as active stimuli for current experimentalists.

At first sight, there seems to be a remarkable diversity in the natures of the various defects which have been suggested. Nevertheless, two elements appear as regular features: the starting-point (i.e. the 'cause') is usually a genetically-determined abnormality expressed in the keratinocyte, and the end-point is an 'explanation' of the increased proliferation which is characteristic of the psoriatic lesion. The great majority of theories differ only in how these two points are bridged.

It is the purpose of this article to bring together certain recent observations which perhaps offer a radically different view. These are as follows:

Langerhans cells may participate in the keratinization process.

Epidermal Langerhans cells are derived from blood monocytes.

Blood monocytes from psoriatic individuals are atypical.

We shall first summarize the evidence regarding each of these points, then discuss the implications for psoriasis and finally outline some possibilities for testing our conclusions experimentally.

THE LANGERHANS CELL (LC) AND KERATINIZATION

A number of authors have speculated that the LC may be concerned in the keratinization process in normal epidermis (see reviews by Sagebiel, Clarke & Hutchens, 1971 and by Wolff, 1972). Such speculation is supported both by ontogenetic observations (Breathnach & Wyllie, 1965; Hashimoto *et al.*, 1966; Sagebiel & Odland, 1971) and by morphological studies (Hutchens, Sagebiel & Clarke, 1971).

Further light may be thrown on the relationship between LC and keratinocytes by the recent work of Green (1978). It has long been known that agents which increase the level of intracellular cyclic AMP (e.g. adrenaline) invariably promote differentiation and reduce proliferation in organized epithelium, either *in vivo* or in culture (reviewed by Voorhees *et al.*, 1974). In a series of elegant and convincing experiments, however, Green (1978) showed that using subcultures of cloned keratinocytes (thus in the absence of LC) precisely the opposite effect is seen. It is difficult to escape the conclusion that the LC releases a differentiation—promoting factor following cyclic AMP-mediated stimulation.

MONOCYTES AND THE LC

The possibility that LC are of mesenchymal origin has been discussed by many early workers (reviewed by Wolff, 1972), but direct evidence has only recently been available. Immunofluorescence

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techniques have since established that the surface marker characteristics of the LC are identical to those of the monocyte-macrophage series (Klareskog *et al.*, 1977); Rowden, Lewis & Sullivan, 1977; Stingl *et al.*, 1977). Very recently absolute proof of the bone-marrow origin of LC has been provided by transplantation experiments (Katz, Tamaki & Sachs, 1979).

MONOCYTE FUNCTION IN PSORIATIC INDIVIDUALS

A number of publications describe changes in bone-marrow derived cells in psoriatic patients. In most cases the extent of the change is related to the activity of the disease, and may simply be a consequence of the cutaneous lesions. However, several groups of workers have now reported alterations of monocyte-macrophage function which seem to be characteristic of the psoriatic individual, being independent of the extent (or even the presence) of cutaneous pathology. These abnormalities include the following:

(a) an enhanced uptake of radioactively labelled blood cells by fixed macrophages (Molin & Reisenstein, 1974)

(b) increased chemotaxis of peripheral blood monocytes (Krueger *et al.*, 1978)

(c) higher phagocytic capacity and bacteriocidal capacity of monocytes (Bar-Eli *et al.*, 1979).

As this last group has pointed out, all these functions are under cyclic nucleotide control, the direction of the change indicating a reduced cyclic AMP/GMP ratio. A low cyclic AMP level has recently been reported in mononuclear leukocytes from psoriatic patients (Geerdink *et al.*, 1980).

IMPLICATIONS

The general outline of our view is now obvious. The primary expression of the 'psoriatic' gene is in the monoblast; this is perpetuated in the promonocyte, the monocyte, the macrophage and finally the LC. The precise nature of this defect, although of course unknown, is manifest as a reduced intracellular cyclic AMP level. Cyclic AMP-mediated functions, which (at the LC stage) include the promotion of differentiation in surrounding keratinocytes, will be altered. In certain—as yet ill-defined—circumstances, the formation of an intact, orthokeratotic horny layer will cease, and a clinical lesion will appear.

From this point of view the two axioms on which most biochemical theories rest are abolished. The consequences are important. First, if the defect is expressed in the bone-marrow rather than the keratinocyte, we must regard psoriasis as a systemic rather than a localized disorder—a concept recently advocated by Mali (1979), who described the disease as a 'destabilisation of the epidermis'. Secondly, if the primary change in the skin lesion is an impaired differentiation, we must interpret the tendency to increased proliferation as the result of the larger germinative cell population. In the established lesion this will, of course, be maintained and exaggerated in response to the chronic, endogenous wound.

Certain unfashionable observations may now be recalled from an undeserved obscurity. In particular, we may note the experiments of Christophers & Braun-Falco (1970), who mimicked the cell-cycle kinetics of the psoriatic lesion by the application of irritants to normal human skin. Despite the fact that the transit time was reduced to 3 days, parakeratosis did not result. We might also remember that Fry & McMinn (1968; 1970), studying healing in psoriatic lesions, showed that proliferation does not diminish until *after* the granular layer has been re-established.

One recent observation must be singled out for especial comment, since it is the only example so far discovered of a clearly-defined enzyme defect characteristic of psoriatic epidermis which is unrelated to disease activity. This is the lack of inducibility of aryl hydrocarbon hydroxylase (AHH)

(Chapman, Rawlins & Shuster, 1979). Since this enzyme system initiates the chemical degradation of many xenobiotics, and since the LC specifically takes up such materials (Shelley & Juhlin, 1977) it seems likely that epidermal AHH is in fact located in the LC. Chapman's data may thus be wholly compatible with a defect in the monocyte-macrophage-LC line.

EXPERIMENTAL APPROACHES

The ideas outlined here, although possibly somewhat iconoclastic, are amenable to direct testing. The major technical problems involve the isolation of reasonably pure preparations of the various cell types. If this can be achieved, each postulate may be tested individually. In particular:

Is there indeed an abnormality of cyclic AMP metabolism in psoriatic monocytes-macrophages?

Is this fault perpetuated in the LC?

Can the influence of LC on keratinocytes be demonstrated *in vitro*? Does this differ using psoriatic LC?

Although peripheral blood monocytes may be isolated using established procedures, and will readily differentiate to macrophages in culture, certain limitations must be recognized. In particular, the attachment/detachment techniques generally employed will certainly cause marked changes in cyclic nucleotide levels. A novel approach might be to employ micro-carrier beads for the selective attachment of monocytes; the suspension may be used for biochemical analysis with the cells remaining *in situ*.

The isolation of LC from epidermis presents greater problems. A preparation enriched in LC has been obtained from trypsinized epidermis by rosetting with IgG-coated erythrocytes followed by isopycnic centrifugation (Stingl *et al.*, 1978). It is probable that this approach could be improved by coupling the IgG to a supporting matrix and using this material as absorbant in an affinity-chromatography system.

The remaining experiments, directed at elucidating the influence of LC on keratinocyte growth, are logically approached by cell culture techniques using cloned keratinocytes. In particular, can keratinization be modified by co-cultivation with intact LC, by the addition of LC extracts, or by 'conditioned medium' derived from LC? In the last case, is the release of the active agent dependent on cyclic AMP stimulation of the LC preparation? And finally—the obvious question—is the course of events modified if the LC are derived from psoriatic patients?

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British Journal of Dermatology (1980) **103**, 461.

Correspondence

Intraepidermal Epithelioma of Jadassohn

SIR, The article by Cook & Ridgway[1] has once again focussed attention on Jadassohn's intra-epidermal epithelioma and the problem of upholding such an entity.

I would like to offer my comments, not subscribing to the view expressed by Cook & Ridgway.

Terminology

The designation 'intraepidermal epithelioma of Jadassohn' is a misnomer.[5,6,17] Borst (1904), Joseph Jadassohn (1926) and (not mentioned by Cook & Ridgway) Werner Jadassohn (1935), have contributed in establishing this entity[5,6,7,8] thereby reflecting the contemporary concept of 'basalioma' and 'spinalioma' as two analogous tumours in juxtaposition to each other within the system of epidermal and appendageal new growths. This concept has been changed in more recent years [5,14,16,17] and, accordingly, one has to be very critical as regards the so-called intraepidermal epithelioma of Jadassohn. I should like to emphasize what Cook & Ridgway[1] say on p. 664 of their article: 'It is doubtful whether Borst or even Jadassohn merit the eponymous use of their names in association with the tumour described here.'

I consider it more appropriate rather to speak about a 'Borst phenomenon', 'Jadassohn phenomenon', and 'Paget phenomenon', when dealing with intraepidermal tumours. [17]

Character of the lesions with intraepidermal proliferations

Mehregan & Pinkus (1964) [10] could demonstrate conclusively that irritating seborrhoeic keratoses by stripping of their surface will lead to the appearance of intraepidermal foci of squamous cells, i.e. part of the lesions presenting with whorls, eddies, etc. undoubtedly belong to this group. Another variety of intraepidermal proliferations and their histopathological characteristics led some authors to assume a point of origin within the acrosyringeal part of the epidermis. In the first place credit goes to Smith & Coburn (1956 and 1957) (Cook & Ridgway[1] consider two of their series of tumours belonging to this group).

I should like to stress that during the late fifties and the sixties a series of papers by different authors dealt with an approach to classification of epidermal and appendageal new growths, by enzyme-histochemistry, the results of which still appeal to me, since no better criteria for systematization of these tumours have been developed.[2,13,22,23] Another series of papers have dealt with the question of histogenesis of intraepidermal tumours in particular, as evidenced by histopathology and histochemistry. These papers, as well as the former ones, [3,4,5,6,9,11,12,15,20,21] are not given credit by Cook & Ridgway.[1]

Histopathology

The microscopic growth pattern called 'acervate' by Cook & Ridgway[1] was described in 1969 and also illustrated by photography ([4] p. 627 and Fig. 2d p. 629; then called 'parakeratotic globules').

I believe that this phenomenon is a reflection of the biologically different growth dynamics of acrosyringeal epithelium vs interfollicular epidermis.

Conclusion

The lesions under consideration here cannot be intraepidermal 'basaliomas' (Holubar[6]). The term 'epithelioma' is imprecise and should be avoided because it is conceived differently in different languages. To consider them as plain keratoses is not appropriate either, given the particular structural details of growth pattern and cell morphology.

Consequently, the most likely explanation for *those lesions* not being seborrhoeic or other keratoses would be to consider them as of acrosyringeal origin, i.e. appendageal in character.

I agree with Cook & Ridgway on the difficulty of finding convincing proof for the nature and biological character (benignity vs malignancy) and, moreover, for the histogenesis of intraepidermal new growths. I do not agree, however, with the re-establishment of an entity the character of which has justifiedly been questioned and the existence of which has been criticized.

Vienna, Austria KARL HOLUBAR

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SIR, The article 'The intra-epidermal epithelioma of Jadassohn: a distinct entity' by Cook & Ridgway[1] refocuses attention on an often discussed subject[2] and introduces a viewpoint that is questionable.

The term 'intraepidermal epithelioma of Jadassohn' is derived from J. Jadassohn's description of an intraepidermal basal cell epithelioma[3] and by omitting the words 'basal cell' it is open to change of meaning. This change was first introduced, to my knowledge, by Graham & Helwig[4] who described 'spotted parakeratosis, hypergranulosis, sometimes horn cysts, plaque-like acanthosis and discrete nests of epithelial cells within the epidermis', a description that deviates greatly from that of a basal cell epithelioma, but fits well the acervate tumours dealt with by Cook & Ridgway. Graham & Helwig and the present authors also agree in reporting several cases of invasive carcinoma, a feature that has not been seen with intraepidermal basaliomas[5].

I believe, therefore, it would be preferable if the term 'intraepidermal epithelioma of Jadassohn' should be either forgotten or restricted to its original meaning of nest formation of basaloid cells in the epidermis. Such cases probably are identical with cases of the Smith-Coburn type[1]. The tumours described by Cook & Ridgway could very aptly be described by the new term 'acervate epidermal tumours' as the authors suggest.

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SIR, We appreciate Professor Holubar's and Dr Pinkus' comments on our paper. Both of these correspondents disagree with the association of the name Jadassohn with out intraepidermal tumour. Dr Pinkus emphasizes that, as we clearly stated in our paper, the original Jadassohn lesion was a basalioma, not an epithelioma. Both authors, however, appear to accept the existence of the entity that we described[1] and Dr Pinkus comments on its similarity to the tumour described by Graham & Helwig.[2] We, also, felt that the name was not ideal but since it had been in frequent use[3] and since we defined our meaning clearly, we felt that that merited its continuation in usage.

As regards the precise nature and origin of our tumour, we accept Professor Holubar's comments on the various studies that have been done on nesting tumours and we support his belief that the tumours we described are probably of acrosyringeal origin.[1] However, we are less sure that his clarity of concept of these epidermal tumours is accepted in Western Europe or the U.S.A. where they have been regarded as variants of seborrhoeic keratoses[4,5] and we felt it important to emphasize on the basis of our study that the histology and cytology is squamoid and that the tumours have the potential to invade and to metastasize[5].

In conclusion, we feel that the essence of the disagreement on the part of both correspondents is on semantics. We would welcome, as Dr Pinkus suggested, the use of the new term 'acervate epidermal tumour' particularly as this leaves the question of cell origin open.

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British Journal of Dermatology (1980) **103**, 465.

Book Reviews

Essentials of Skin Disease Management. By D.J.Barker and L.G.Millard (1979) Oxford: Blackwell Scientific Publications. Pp. 85, 6 illustrations. Price £3.75.

The authors emphasise that this is a pocket book of management for non-specialists and as such it is concise, easily read and practical. There are brief outlines of the principles of treatment, details of simple remedies and a selection of proprietary preparations. Anyone who practises dermatology for a livelihood will, as he reads it, think of improvements, changes or alternative remedies, but a fair judge will admit that most of these suggestions will be minor quibbles. A few criticisms can be argued more strongly, for example podophyllin made up in tinc.benz.co. stays where you want it much better than that in a spirit base; a griseofulvin dose of 500 mg a day is too low for many adults and gentamicin is surely so precious in the treatment of septicaemia that we should forgo it on the skin. I suspect it is a measure of the general good sense of the book that one wants to argue all these points with the authors. What a pity that Blackwells could not manage a real best-seller price. it deserves to be widely used and can safely be recommended to all. Perhaps some wise pharmaceutical company will buy in bulk and hand out Barker and Millard's instead of ball-point pens or free lunches. I hope they do.

S.O.B.Roberts

Dermatology for the Physician—an illustrated guide. By E.L.Rhodes. Baillière Tindall. 104 pp. 163 col. illus. Paperback. Price £8.50.

According to the preface, this book is designed primarily for the medical student and the general physician so it is necessary for the reviewer to approach his task within this constraint. It is essential that a book for such a readership should have a large number of illustrations since even today there are many who, when confronted with a patient having a rash, turn the pages of a well established atlas until he can triumphantly shout 'snap'. On the whole, the illustrations are adequate but small, some are repeated and some are not numbered to correspond with the captions.

Inevitably in a book of this size, only thumb-nail sketches can be given of the diseases discussed and inaccuracies often of ommission creep in. Yaws is not confined only to Africa, India and Northern Australia; alopecia areata does not always respond to corticosteroid therapy; ringworm does not give complete baldness but the affected hairs are broken short and the medical student should be reminded that small spored ringworm does not normally occur after puberty The dangers of podophyllin in vulval warts in pregnancy should be stressed; bullous pemphigoid is uncommon before the age of 60 and pityriasis rosea affects mainly the trunk.

These random criticisms are minor in a book which attempts to cover a very wide subject concisely but they are points in diagnosis which any student or general physician should be told.

In revising the book, the expression 'of course' which occurs repeatedly should be banished. It gives the impression of a verbatim report of a lecture and although may be easy to listen to can make dull reading. In addition, since the book was constructed from a series of separate articles, there is a certain amount of unnecessary repetition.

In spite of all the above, the book has achieved its objects and has a place in a medical library.

Renwick Vickers

Essentials of Dermatology. By J.L.BURTON. Churchill Livingstone Edinburgh: Pp. 186. Price £3.95. This is the latest of a number of short text books on the subject intended to be useful to undergraduate students and those practising general medicine. Its author has a very high reputation as an eminent clinician and research worker, and the book is based on his lectures to students at Bristol University.

The text covers the subject widely enough and concisely enough to be fully comprehensive for its purposes, and the bibliography is appropriate. It reveals its author's sense of humour and this makes for very easy reading and appeals to undergraduates whose texts are all too often dull and wordy. The excellent drawings and diagrams (mainly the work of the author himself) are more illustrative of the text than photographs would be, and have been favourably commented on by readers. The whole style and production of this book makes it possible to recommend it wholeheartedly and it should prove a great success.

OLIVER SCOTT

Immunopathology of the Skin. Edited by E.H.BEUTNER, T.P.CHORZELSKI and S.F.BEAN (1979) New York: John Wiley and Sons, Price £23.80.

The first edition of this book established a well justified reputation of indispensibility amongst students of cutaneous immunopathology. The subject is developing fast and the editors have taken this opportunity not only to up-date but also to re-shape the book.

The section on syphilis has been omitted because most dermatologists now no longer deal with venereology and other sections have been expanded. The emphasis of the book has been changed slightly and it is now orientated more towards practising clinicians.

The book consists of four main sections: indications, methods and concepts for immunopathologic studies, bullous disease, connective tissue disease and other labelled antibody findings in immunopathologic studies of the skin.

Those wishing to set up an immunofluorescence service will find there are sufficient data in the first section, together with the major pitfalls and problems they are likely to encounter. Detailed attention is paid to methods for preparation of reagents and testing the specificity of conjugates. However, problems with substrates may be encountered by those unable to obtain regular supplies of suitable monkey oesophagus.

The remaining sections bring the bullous, connective tissue and related disorders right up-to-date with good readable accounts and very complete references. Growing points such as herpes gestationis, linear IgA dermatoses, mixed connective tissue disease and the lupus band test have been given more prominence in this edition and are well discussed.

This book should be available for reference in all departments of dermatology and I would strongly recommend that it should be read by dermatologists in training.

RICHARD J.PYE

Antifungal Chemotherapy. Edited by D.C.E.SPELLER. New York: John Wiley & Sons. Pp. 446. Price £28.00.

Contributors to this book include microbiologists, medical mycologists, clinicians, a medical laboratory scientist, and a pharmaceutical research worker: thus the subject of antifungal chemotherapy is covered from several angles. The book is divided into three parts.

The first part discusses antifungal drugs which are used systemically. The chemical and physical properties, mode of action, laboratory studies and pharmacokinetics are dealt with in detail and there is much of interest for the scientist. Of more immediate use for the clinician are the sections on resistance, drug interactions and the use of antifungal drugs in human mycoses. Dermatologists will find much of practical use in the comprehensive chapter on the treatment of superficial and subcutaneous mycoses, which includes the wise, but often neglected advice to actually explain to the patient how to apply his cream. Although systemic mycoses are rare in this country, migration of populations means that British medical practitioners may, on occasion, meet with one. They will find that the chapter relating to these diseases contains concise information on geographic distribution and clinical and laboratory diagnosis, as well as management. The section ends with two chapters on different aspects of opporcunistic fungal infections, a growing problem with the increased use of immunosuppressive drugs.

The final section of the book on laboratory control will be of most use to the microbiologist, but will also interest clinicians who do their own mycology.

Each section is followed by a full list of references and the index picks out in capitals the most important diseases and drugs.

'Antifungal Chemotherapy' should find a place in most medical and laboratory libraries but the clinician with an interest in mycology will probably want to have his own.

M.M.WALSHE

Handbuch der Haut- und Geschlechtskrankheiten. Normale und Pathologische Physiologie der Haut II. By. J.JADASSOHN. Berlin: Springer Verlag. Pp. 606, 196 Abb., 80 Tab. Price DM580.00, US $319.00.

We have come to regard the handbook series as being the repository of all relevant cutaneous knowledge. Although this may not be entirely true, the series certainly serves to bring together and discuss most of the important facts in a particular field. The present volume is a treatise on aspects of the physiology and pathophysiology of the skin. Keratinization is dealt with *in extenso* and there is an excellent section on physiology of the stratum corneum. I was also pleased to see that the volume includes many newer techniques which I sincerely hope will be moved into the clinic in the near future. There are also comprehensive sections on neurophysiology and sweating which contain some striking photographs of sweat patterns. As with previous volumes, the book is copiously referenced and contains many excellent illustrations and photographs. It is to be highly recommended as a serious work of reference.

R.MARKS

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News and Notices

The Second International Dermatopathology Symposium, London, 12–15 July 1981

The Second International Dermatopathology Symposium, entitled 'Dilemmas and Directions in Differential Diagnosis' will be held at Grosvenor House Hotel, Park Lane, London, 12–15 July 1981. The symposium is co-organized by Professor E. Wilson Jones and Dr Martin Black of the Institute of Dermatology, University of London, and Dr A.Bernard Ackerman of the Section of Dermatopathology, New York University School of Medicine. The emphasis will be on histological clues to diagnosis by conventional microscopy, clinical, electron microscopic and immunopathological aspects of inflammations, neoplasms, malformations and deposits of abnormal materials in the skin.

The Faculty includes Drs W.Gebhart, K.Holubar, H.Kerl, G.Stingl from Austria; Dr. D.Weedon from Australia; Dr G.Pierard from Belgium; Drs M.M.Black, S.S.Bleehen, R.A.J.Eady, K.Henry, M.Hutt, I.Kramer, D.M.MacDonald, D.H.McGibbon, R.A.Marsden, T.J.Ryan, N.P.Smith, E. Wilson Jones from England; Dr J.H.Saurat from France; Drs P.Braun-Falco, G.Burg, L.Leder, G.Plewig, H.Wolff from Germany; Dr R.Caputo from Italy; Dr J.Sanchez from Puerto Rico; Drs J.A.A.Hunter, R.M.MacKie from Scotland; Dr J.M.Mascaro from Spain; and Drs A.B.Ackerman, M.Chalet, W.H.Clark, Jr., J.Grant, J.H.Headington, E.Heilman, R.Jones, W.F.Lever, J.Maize, M.Mihm, J.Patterson, H.Pinkus, A.Ragaz, T.Wade, C.White, R.K.Winkelmann from the United States.

All enquiries should be addressed to: Marcus Summersfield, London Symposium 1981, Conference Co-Ordinates, Regent House, 60 King Street, Twickenham, Middlesex TW1 3SH, with the exception of specific enquiries regarding the medical aspects of the programme which should be addressed to: Professor E. Wilson Jones, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ.

Whimster Prize

Following the suggestion of many of the late Ian Whimster's friends and colleagues, the Executive Committee of the Association plans to establish a prize in his honour.

A small committee has been formed to recommend the Regulations to govern the award of this prize and anyone wishing to make suggestions is invited to write to the Honorary Secretary of the British Association of Dermatologists, 7 John Street, London, WC1N 2ES on the matter.

Fourth Ain Shams Medical Congress, March 14–16 1981

Ain Shams Faculty of Medicine, Cairo, announces its 'Fourth Ain Shams Annual Medical Congress' to be held in Cairo, 14–16 March 1981. The main theme of the congress will be 'The Skin'. A half day clinical meeting will be included. Members of the Dowling Dermatology Club of London will be actively participating in this congress. Abstracts from other participants should be received before 30th December 1980.

Application forms for detailed information will be sent on request. Please write to: Professor Aly Abdel-Fattah, Secretary General of Ain Shams Medical Congress, Faculty of Medicine, Abbassia, Cairo, Egypt.

Advanced Seminar. Recent Advances in Clinical Dermatology

This annual seminar is intended for Senior Registrars and Consultants in Dermatology. It is expected that sessions will include Histopathology, Immunopathology, Infections, Malignancy, Bullous Diseases, Contact Dermatitis, Pigmentary Disorders, Genodermatoses, Psoriasis and Photodermatitis. Maximum possible use will be made of volunteer patients and of illustrative material. The Course will be held from

6–10th April 1981 at The Institute of Dermatology, and St John's Hospital for Diseases of the Skin. Details can be obtained from The Dean, Institute of Dermatology, 5 Lisle Street, London WC2H 7BJ.

12th International Congress of Chemotherapy

This Congress will be held in Florence, Italy, July 19th–24th 1981. Topics will include antimicrobial, anticancer and antiviral chemotherapy, as well as immunology and immunotherapy. Deadline for abstracts is February 15th 1981, and further information may be obtained from: Secretariat, 12th International Congress of Chemotherapy, Via della Scala, 10, Florence, Italy. (Florence 50123.)

XVI Congress of Dermatology—1982

This will be held at the New Otani Hotel in Tokyo, 23rd to 29th May 1982. The following subcommittees have been appointed for the Scientific Sessions.

1. Case Presentations: Profs. Wilkinson (Chairman), Civatte (Co-chairman), Cordero, Kukita.
2. Special Lectures: Profs. Seiji (Chairman), Baer (Co-chairman), Wilkinson, Wolff.
3. Advances in Dermatology: Profs. Winkelmann (Chairman), Braun-Falco (Co-Chairman), Dominguez Soto, Seiji, Wolff.
4. Symposia: Profs. Jablonska (Chairman), Padilha Gonçalves (Co-chairman), Braun-Falco, Cabré, Kukita.
5. Workshops: Profs. Wolff (Chairman), Jablonska (Co-chairman), Civatte, Cordero, Serri, Seiji.
6. Courses: Profs. Civatte (Chairman), Winkelmann (Co-chairman), Kukita.
7. Free Communications: Profs. Serri (Chairman), Thyresson (Co-chairman), Cabré, Yasuda.
8. Informal Discussion Groups: Profs. Dominguez Soto (Chairman), Padilha Gonçalves (Co-chairman), Seiji, Yasuda.
9. Posters: Profs. Cordero (Chairman), Thyresson (Co-chairman), Seiji, Yasuda.
10. Scientific Exhibition and Audiovisual Communications. Profs. Yasuda. (Chairman), Kukita (Co-chairman), Seiji.
11. Seminars for Asian Dermatologists. Profs. Yasuda (Chairman), Kukita (Co-chairman), Seiji.

The following working hours were decided: Morning session 8.30–12.30; Afternoon session 14.00–18.00. Workshops and Free Communications would be given in the afternoon sessions.

It is proposed to publish the abstract and proceedings of the Congress as soon as possible after this takes place.

The Executive Committee of the British Association of Dermatologists have asked Stiefel Laboratories Limited to explore the possibilities in regard to travel arrangements as part of the Stiefel Travel Service.

Case Presentations. Dr D.S.Wilkinson is very anxious to obtain suitable material for consideration of presentation at this very popular feature of the Congress. Members are earnestly invited to collect such material and are invited to contact Dr Wilkinson directly at any time during the next twelve months. The final date of submission of material is Summer 1981.

Stanford Third International Symposium on Psoriasis

Stanford University's Department of Dermatology is presenting the Third International Symposium on Psoriasis, to be held July 13–17, 1981 at Stanford University. Significant advances in psoriasis research and therapy will be emphasized through plenary sessions, workshops (free communications) and poster exhibits during which opportunities for direct discussions between investigators and attendees will be provided. Campus housing is available at special rates, in addition to motel and hotel accommodations in the area. Several tours are planned for the enjoyment of symposium attendees and their spouses. For further information, please contact: Paul H.Jacobs, M.D., Department of Dermatology, Stanford University School of Medicine, Stanford, California 94305, U.S.A.

Preliminary Announcement. The Stratum Corneum—an International Symposium

An International Symposium on the stratum corneum will be held at the Welsh National School of Medicine, Heath Park, Cardiff, on the 29th, 30th and 31st October 1981. There will be invited lectures, free communications and poster papers. Abstracts should be submitted by the 26th June 1981 in the style as submitted to the European Society for Dermatological Research. We believe this symposium will be of importance for dermatologists, toxicologists, cosmetic scientists, the pharmaceutical industry and others interested in skin biology. Organizing committee: Dr R.Marks, Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN; Dr G.Plewig, Dermatologische Universitätsklinik, Frauenlobstraße 9-11, D-8000 Munchen 2. Further information can be obtained by contacting Dr R.Marks.

APPOINTMENTS

West Berkshire Area Health Authority

Dr Martin James has been appointed to the post of Consultant Dermatologist at the Royal Berkshire Hospital, Reading.

His undergraduate medical training was undertaken at St Thomas' Hospital, London where he was awarded B.Sc. (first-class Honours in Anatomy) in 1969, and graduated M.B., B.S., in 1972. M.R.C.P. (U.K.) was obtained in 1974. He became Registrar to the Dermatology Department of St Thomas' Hospital in 1975 and later became Senior Registrar at St John's Hospital for Diseases of the Skin. His most recent post was Senior Registrar to the Department of Histopathology at St John's Hospital.

He has published a number of clinical and scientific papers but his main interest has been the morphological changes occurring in the physical urticarias and experimentally-induced acute inflammatory reactions in man.

Grampian Health Board

Dr Marion I.White has been appointed Consultant Dermatologist to the Grampian Health Board. Dr White studied medicine at Aberdeen University graduating M.B., Ch.B in 1972. She worked in general medicine in Cambridge and Glasgow, proceeding M.R.C.P. in 1974. She studied pathology in Glasgow before commencing training in dermatology in Aberdeen in 1975. Since January, 1979, she has held a M.R.C. research fellowship at St John's Hospital for Diseases of the Skin, working on a project to define the effects of bacterial colonization on the skin.

St Helens and Knowsley and Liverpool Area Health Authorities

Dr Susan Evans has been appointed Consultant Dermatologist to the St Helens and Knowsley and Liverpool Area Health (Teaching) Authorities. She is married to a Consultant Physician and has three children. She qualified M.B.,Ch.B. at Liverpool University in 1964. After a year's Psychiatry she began working in the University Department of Dermatology in 1966, first as a Research Fellow and later as a Lecturer.

Her main interests are in Cancer and Chemical Carcinogens, and she obtained her M.D. in 1976 on Arsenic and Cancer. She has published several papers on diverse aspects of Dermatology and continues her interests in carcinogenesis and University teaching.

Gloucestershire Area Health Authority

Dr J.M.Boss has been appointed to the post of Consultant Dermatologist to the Gloucestershire area.

Dr Boss graduated M.B., Ch.B., Birmingham in 1972 and obtained his M.R.C.P. (U.K.) in 1975. Following posts in General Medicine in Birmingham and Stoke on Trent he became registrar in Dermatology at Stoke on Trent. Since 1978 he has been Senior Registrar in Dermatology at Bristol Royal Infirmary.

His publications have been mainly of a clinical nature and he has a particular interest in the pharmacological effects of histamine antagonists.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	µg	cubic millimetre(s)	mm^3	micrometre(s)	µm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a,) b. All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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British Journal of Dermatology

The British Journal of Dermatology is owned by and is the official organ of the British Association of Dermatologists. It is also the official organ of the Nederlandse Vereniging van Dermatologie en Venereologie (Netherlands Association for Dermatology and Venereology).

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REFERENCE:
British Medical Journal, 1978, **2**, 84.

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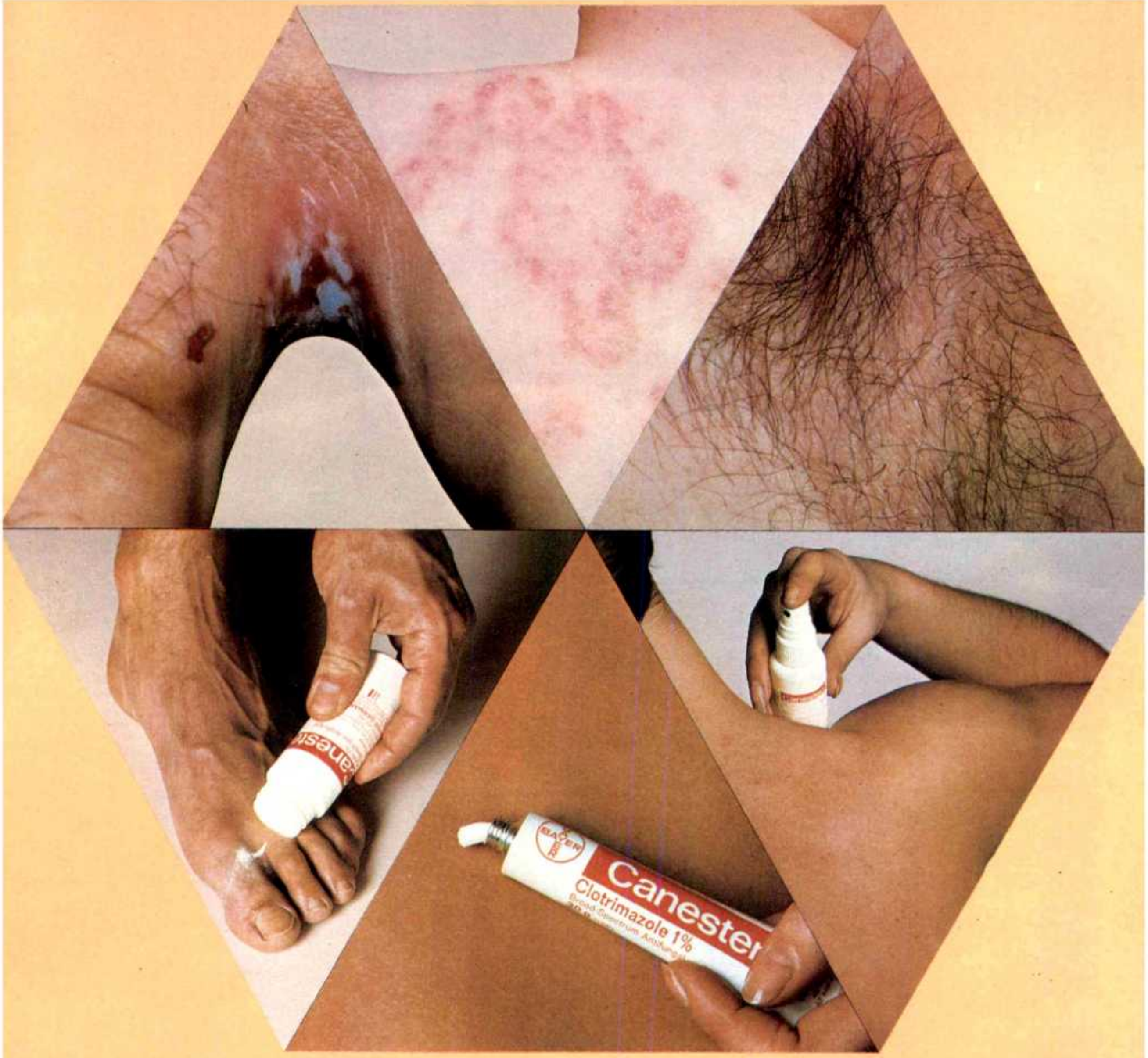

In fungal infection Canesten® covers all the angles

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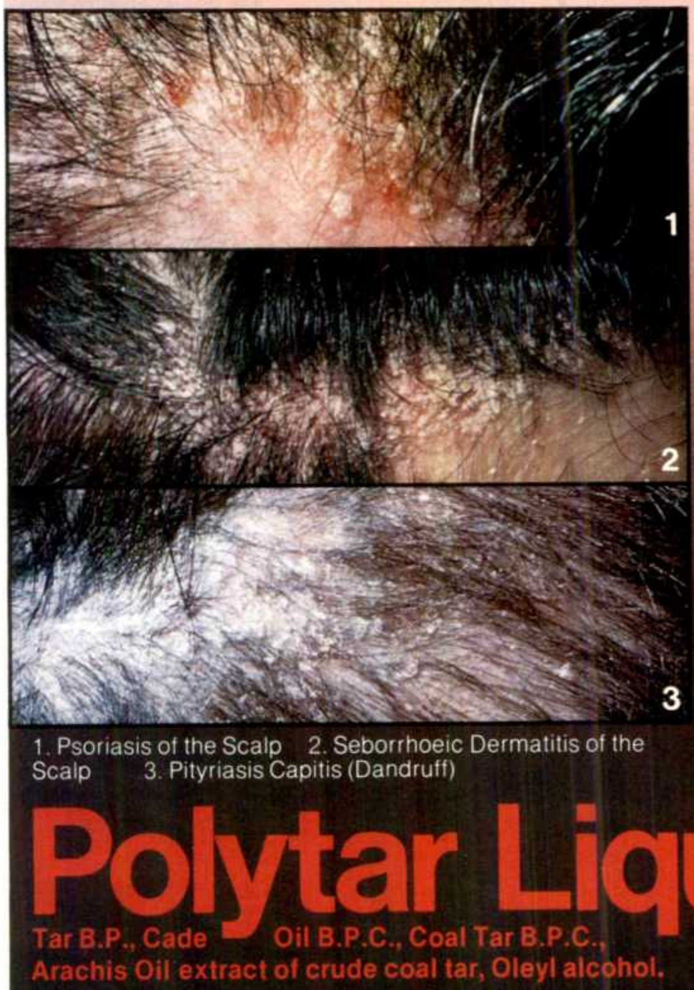
Common Disorders of the Scalp.



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Pharmaceutical Precautions Store in a cool place

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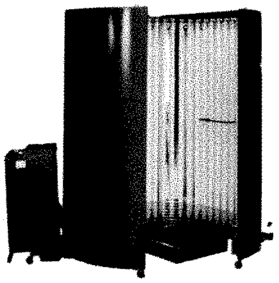

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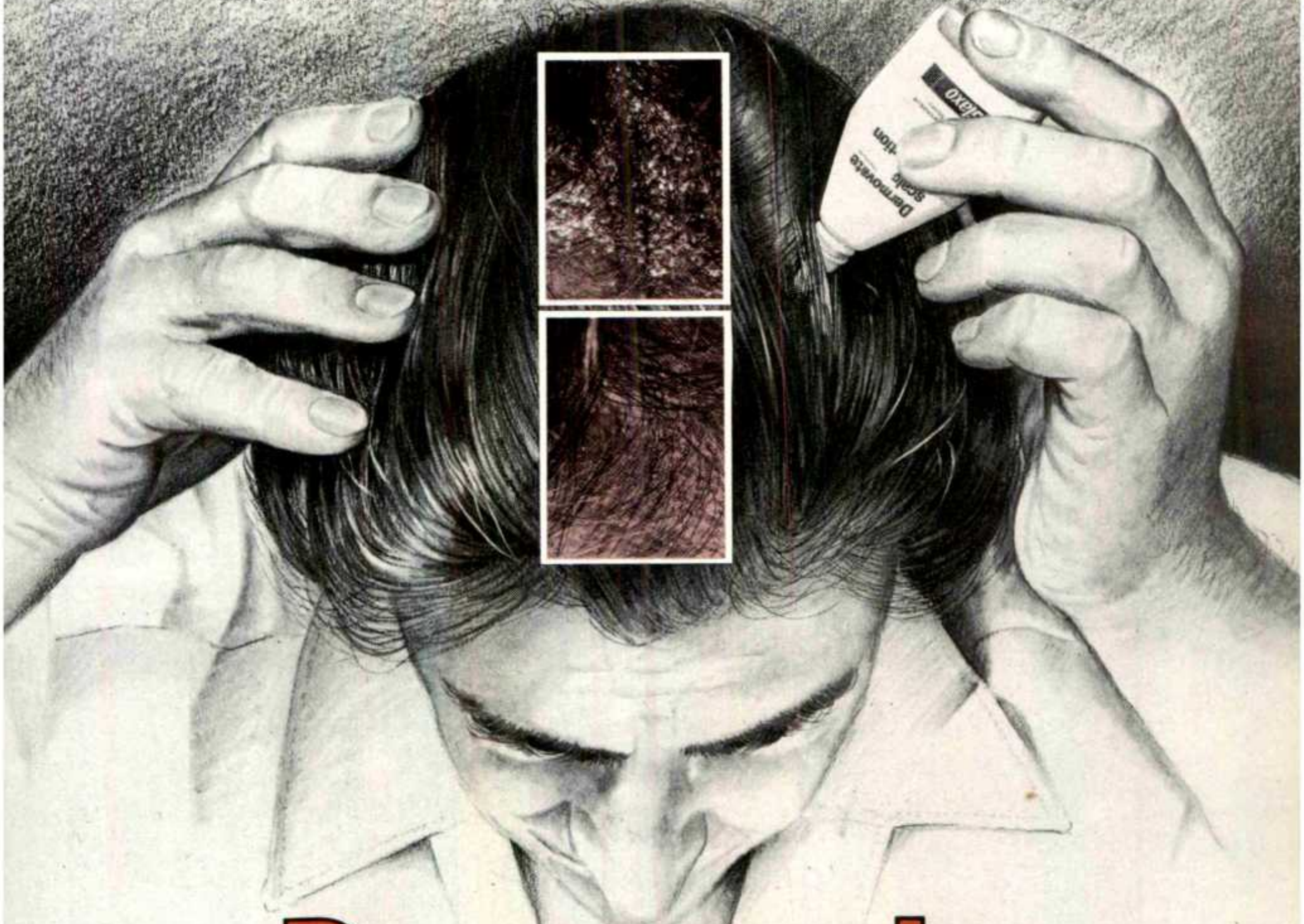

Dermovate

(clobetasol propionate)

Scalp Application

Prescribing information

Uses

Psoriasis and recalcitrant eczemas of the scalp. Clobetasol propionate is a highly-active topical corticosteroid which is indicated for use in short courses for conditions which do not respond satisfactorily to less active steroids.

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Precautions

Care must be taken to keep the preparation away from the eyes. Do not use near a naked flame. Long-term continuous therapy with Dermovate Scalp Application should be avoided, particularly in infants and children, in whom adrenal

Development of secondary infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic antimicrobial therapy.
Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development.
In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

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Dermovate preparations are usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. As with other topical corticosteroids, local atrophy may result from prolonged treatment, particularly if occlusive dressings are used. When extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism, especially in infants and children. This effect is more likely to occur after prolonged treatment, particularly if occlusive dressings are used. In rare instances, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have

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✱Highly acceptable(4)

Locoid C

Hydrocortisone 17 butyrate 0.1% chlorquinaldol 3.0%

When infection is present or suspected.

References:

(1) Amer Pract. & Digest of Treat (1955) 6 8
(2) Transact New York Acad of Sci (1955) 10 161
(3) Arch Derm (1972) 106 335
(4) Rocky Mtn Med J (1955) 52 6

Prescribing Notes

Dosage & Use. Apply to the affected part two to four times a day until the infection is resolved. If the underlying condition persists treatment may be continued for up to 14 days and no longer. No more than 60g may be applied during this period. Do not use under occlusion in the presence of infection.

Contra Indications. As with all topical steroids, Locoid C preparations are contraindicated in the presence of viral, tubercular or syphilitic lesions.

Other Precautions. Avoid contact with the eyes. Not to be used in pregnancy in large amounts or for long periods.

In infants, avoid long term continuous therapy, especially avoid occlusion.

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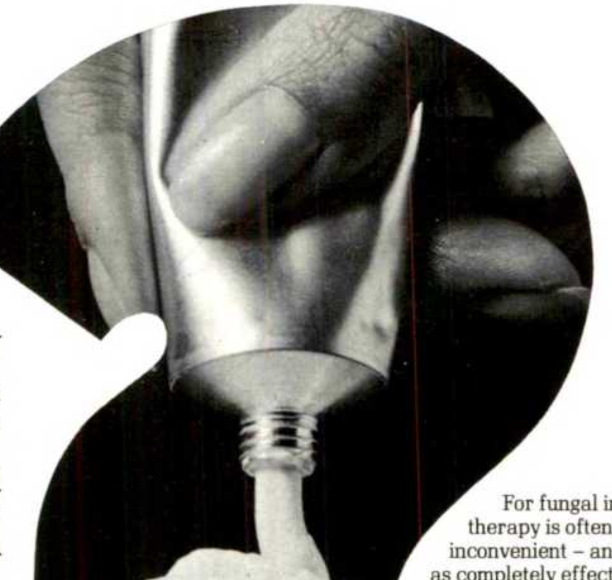

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Prescribing information

Dosage and administration

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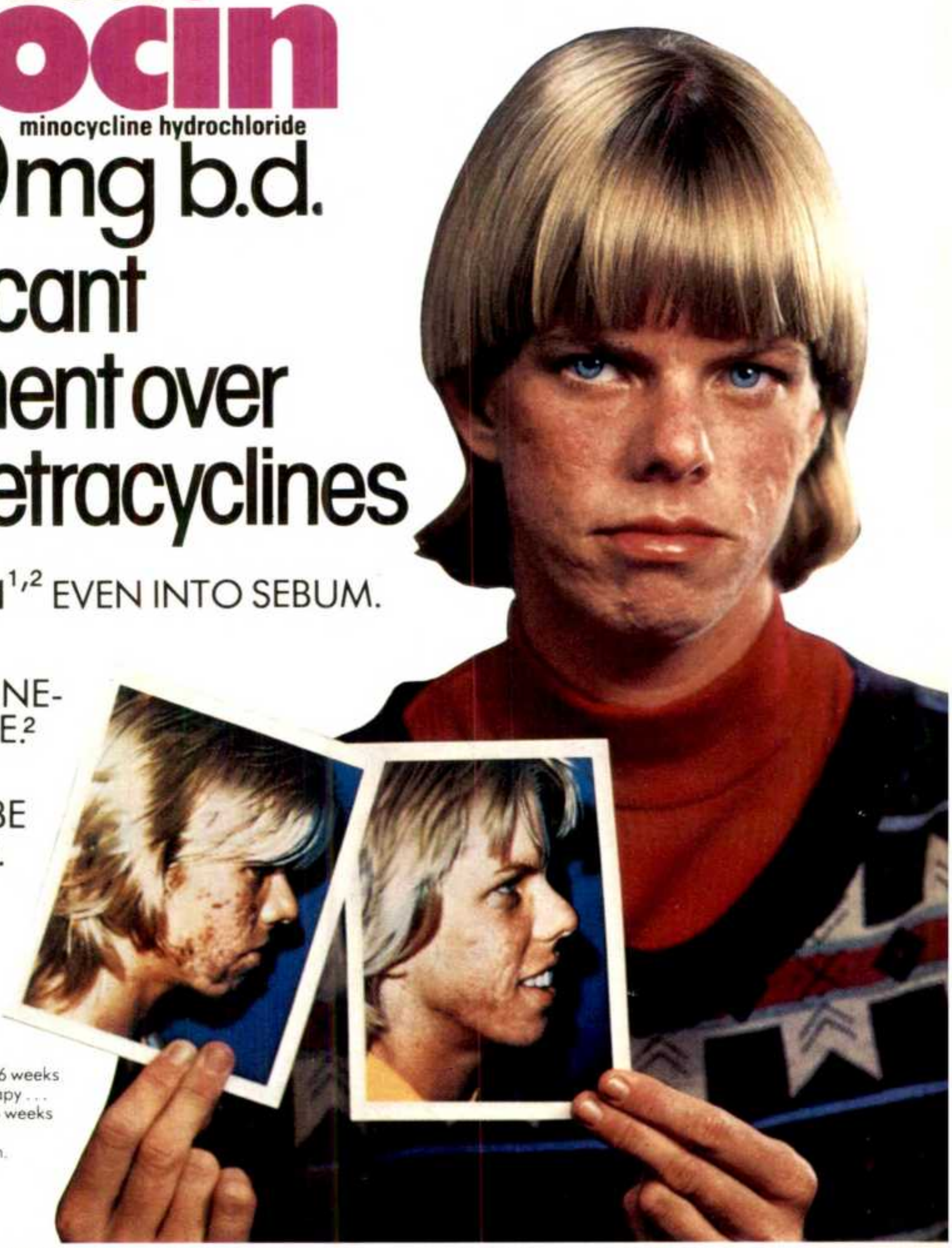

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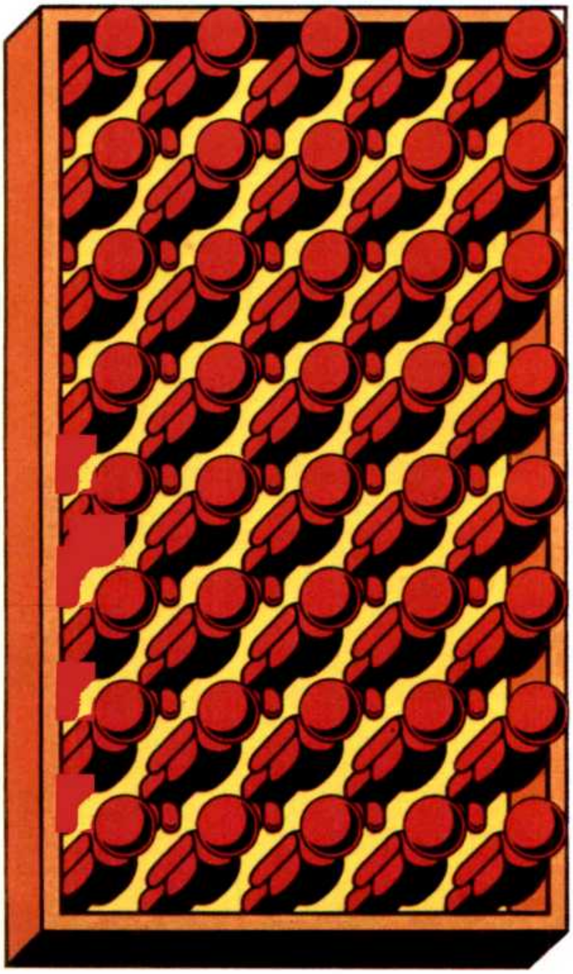

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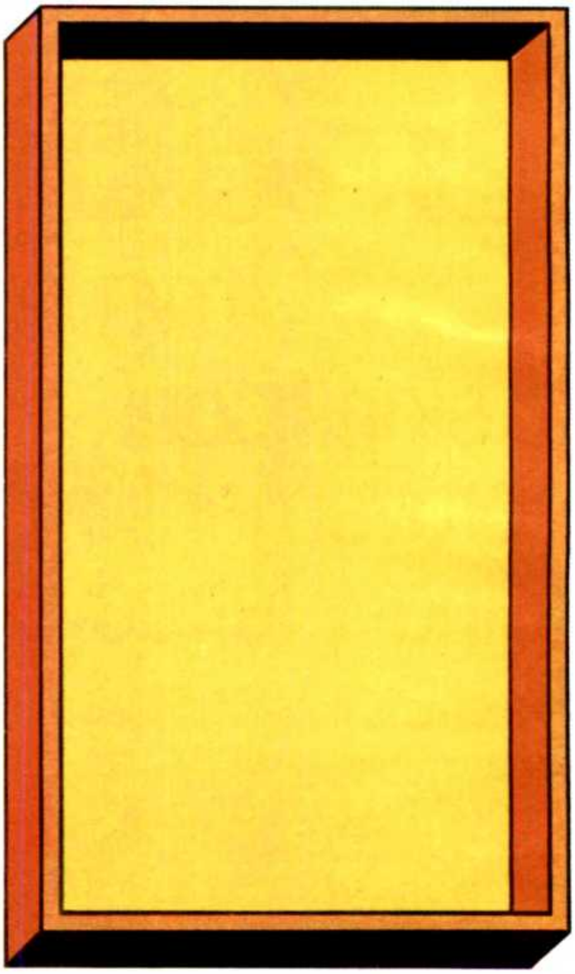

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1. *British Journal of Dermatology*, 1979, **100**, 475

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Clinical and Laboratory Investigations

Oral retinoids—present status

C.E.ORFANOS

Professor and Chairman, Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, 1, Berlin 45, West Germany

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Oral retinoids are synthetic derivatives of vitamin A (retinol); they are potent and rather specific dermatotherapeutic drugs. Their development in the last 10 years and their clinical application has clearly introduced a new era in dermatotherapy. Skin diseases resistant to traditional local remedies, such as pustular psoriasis and Darier's disease and other keratotic genodermatoses, obviously respond to oral retinoid treatment. Additionally, since retinoids exert a particular pharmacological effect on skin and its appendages as a target tissue, their use *in vitro* and in animal experiments may help to increase our knowledge about the mechanisms of keratinization and epidermal growth control.

Biosynthesis of new drugs often results from empirical knowledge of basic compounds, possibly of natural origin, indicating some beneficial properties in certain pathological conditions. In this case vitamin A was known to improve hyperkeratotic conditions in general, including acne; however, its therapeutic use was limited by its toxicity. Its therapeutic ratio, i.e. the relation between therapeutic dose and the lowest toxic dose, seemed inappropriate for long-term therapy in humans. Moreover, possible target tissues for serious side-effects of the original vitamin A include non-keratinizing epithelia of the eyes and the gonads.

In the late sixties and the following years extensive pilot studies with oral application of all-trans-retinoic acid, 13-cis-retinoic acid, all-trans-retinoic acid ethylamide and anhydroretinon acid were undertaken by our group, searching for a new effective compound for systemic treatment with a low incidence of side-effects. The overall results were rather moderate, particularly in psoriasis (Orfanos *et al.*, 1972; Runne, Orfanos & Gartmann, 1973). Similar to Schumacher & Stüttgen (1971) we found that the natural metabolite all-trans-retinoic acid was notably effective in disorders of keratinization and acne; however, in its therapeutic ratio it did not differ significantly from vitamin A. The daily dose required for treatment (50–200 mg) caused, as a rule, considerable side-effects. Anhydroretinon acid was less effective, retinoic acid ethylamide influenced Darier's disease (Orfanos, Kurka & Strunk, 1978a) and 13-cis-retinoic acid was found beneficial for acne and psoriasis at 50–100 mg/d; nevertheless, the frequency of its possible side-effects was still considered too high for it to be recommenced for routine therapy. After administration of retinoic acid ethylamide and 13-cis-retinoic acid in psoriasis elevation of blood sedimentation rate, transaminases, urea and serum lipid levels were noted (Runne *et al.*, 1973). During these studies it became evident, however, that synthetic retinoids did maintain the basic efficacy of the parent compound, whereas some of the typical side-effects such as headaches, nausea and vomiting were less pronounced or rare; in low dosage their action was restricted mainly to keratinizing epithelia and their side-effects were dose-dependent and reversible.

Guest Lecture, given at the Annual Meeting of the British Association of Dermatologists, Oxford, July 1980.

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In 1975 a new aromatic retinoid (Ro 10-9359) became available and some promising preliminary results were observed in psoriasis (Ott & Bollag, 1975; Orfanos & Runne, 1976; Schimpf, 1976). On this basis a multicentre study was conducted and in 1977 the findings of our cooperative team from seven Dermatology Departments in Germany were communicated at the International Congress of Dermatology in Mexico City, on 291 patients with psoriasis treated orally with aromatic retinoid compared to local dithranol treatment (Goerz & Orfanos, 1978). In this multicentre study about 61% of 203 patients with psoriasis responded well, or excellently, to oral aromatic retinoid; some of those who responded cleared completely. Long-term follow-up revealed that about 15% of all patients remained free of lesions for longer than 6 months. Although no separate evaluation related to different clinical types of the disease was undertaken in this study it seemed that particularly severe types of psoriasis responded surprisingly well to oral retinoid treatment. Also, in palmoplantar pustulosis (Fredriksson & Pettersson, 1978; Fredriksson, 1978) and in erosive mucosal lichen planus (Schuppli, 1978) treatment with aromatic retinoid was followed by rapid improvement.

In the last few years these findings have been confirmed by several investigators (Lassus, 1980), and oral retinoids have been shown to possess spectacular therapeutic properties in a number of skin conditions including psoriasis, lichen planus, Darier's disease, ichthyosis, pityriasis rubra pilaris, erythrokeratoderma variabilis, plantar warts, acne, etc. A new broad spectrum oral dermatotherapy seems to have become a reality and to replace systemic corticosteroids and cytostatics (Orfanos, 1979).

CLINICAL EFFICACY

Psoriasis

In psoriasis the efficacy of retinoids seems well established now. All investigators agree that pustular psoriasis, the localized palmo-plantar pustulosis and the generalized type of Zumbusch as well, represent a main indication for oral treatment with aromatic retinoid (Orfanos, Landes & Bloch, 1978b; Chandon *et al.* 1978; Fredriksson, 1978; Fredriksson & Pettersson, 1979). The regression of pustules in psoriasis is one of the most impressive and reliable effects seen with oral intake of retinoid, comparable to the regular appearance of cheilitis.

In pustular psoriasis treatment has to start with a rather high dosage (75 mg/d); the pustules dry out in 2–3 days and disappear in one week on average. They reappear if oral medication is discontinued or reduced before 3–4 weeks. A maintenance dose has to be administered for 2–3 months or even longer. Histological examination shows that the pustules disappear rapidly and typical features of psoriasis vulgaris appear after 2 weeks; after 4 weeks most tissue changes return to normal.

In erythrodermic psoriasis the results are not as dramatic and predictable as in pustular types; however, oral aromatic retinoid is definitely effective in most cases. Rather low doses are preferable (25–35 mg/d), increasing after 3–5 weeks up to 50–60 mg/d. Long-term oral treatment combined with non-irritant local remedies is required, to avoid provocation of new lesions.

Surprisingly enough, chronic psoriasis vulgaris is the type most resistant to retinoids, compared to the response of the pustular types. There is, nevertheless, a highly significant preference for Ro 10-9359 compared to placebo, in studies using 100 mg/d (Lassus, 1980). Oral retinoids can be used alone or in medium dosage (50 mg/d) as a basic or adjuvant drug to enhance the effect of other routine antipsoriatic agents such as local application of dithranol, UVB radiation, PUVA systemically (Orfanos & Runne, 1976; Orfanos *et al.* 1978c; Orfanos *et al.*, 1979b,c; Fritsch *et al.*, 1978) or PUVA locally (Michaëlson, Noren & Vahlquist, 1978). Also combined treatment with Ro 10-9359 in low dosage and triamcinolone acetonide cream topically was found better than topical treatment with triamcinolone alone (van der Rhee, 1980). Histological restoration of most changes, including those in the dermal vessels, occurs after long-term treatment.

Ichthyotic conditions

Ichthyotic conditions in general are a traditional indication for therapy with vitamin A and its derivatives, regardless of their pathogenetic origin and type. All preparations seem to have a beneficial temporary effect in reducing hyperkeratosis, as long as the drug is given.

The oral administration of aromatic retinoid seems to be more appropriate for long-term treatment, due to its lower toxicity. According to our present experience 25–35 mg/d or 35–50 mg every second day is a sufficient dose to maintain remission in vulgar or congenital ichthyosis in adults. Even in early childhood the drug is tolerated well; however, there has been no long-term follow up to exclude damage to bone growth and the gonads. Interestingly, 1–2 mg/kg body weight were given to children, and encouragingly showed no side-effects; these observations suggest that children may metabolize the drug more adequately and avoid toxicity.

Histopathology in lamellar and in X-linked ichthyosis shows reduction of hyperkeratosis with some thickening of the granular layer (Pehamberger, Neumann & Holubar, 1978). In congenital ichthyosiform erythroderma of the bullous type, erythema and bullae may persist or even increase in size and number (Dinet *et al.*, 1978).

Pityriasis rubra pilaris shows rather moderate results with both aromatic retinoid and 13-cis-retinoic acid (Schimpf, 1976; Stüttgen, Ippen & Mahrle, 1977; Peck *et al.*, 1978). In two cases treated by us only low doses (10–25 mg) were well tolerated with a good response after 8 weeks.

In erythrokeratodermia figurata variabilis a marked improvement with all-trans-retinoic acid was reported in three sisters (van der Wateren & Cormane, 1977) and similar results were achieved with aromatic retinoid (Dinet *et al.* 1978; Fritsch, 1979). No side-effects were seen after long-term treatment of a 13-year-old boy with 25 mg/d of aromatic retinoid.

Darier's disease

Darier's disease has been considered in the past as some type of vitamin A deficiency syndrome. Therefore, vitamin A and some of its synthetic derivatives were administered early on and some improvement was seen (Schumacker & Stüttgen, 1971; Günther, 1975a; Peck *et al.*, 1978). Not all cases responded, however, and some relapses occurred, particularly in the summertime, despite maintenance treatment.

The aromatic retinoid seems to be clearly superior to the previous derivatives in this respect (Orfanos *et al.*, 1978a). We are now supervising eleven cases of Darier's disease and in all but one of these cases a rather dramatic response occurred with the aromatic compound. Some erosive and eczematous irritation may occur ofter onset of treatment, particularly in cases treated with higher doses (75 mg/d); in one case new scaly lesions appeared under treatment in non-involved skin areas of the trunk, showing histologically a peculiar pattern of psoriasis-like epidermal changes with some dyskeratosis. Histological examination of lesions responding to oral treatment shows gradual improvement of acantholysis, dyskeratosis and hyperkeratosis in this order. According to these experiences Darier's disease is a main indication for oral treatment with aromatic retinoid. The drug should be given in a medium dose (50 mg/d) in order to avoid initial discomfort for the patient and this dose is lowered to maintenance levels after 3–4 weeks (25–35 mg/d). Two of six cases controlled over a one year period did not relapse after discontinuing the drug; the other patients required continuous treatment in order to prevent recurrence.

Acne

In acne vitamin A has been largely used for several decades in Europe. As a rule, 150,000–200,000 IU were recommended daily, with a low incidence of side-effects (Kalkoff & Bickhardt, 1976). These experiences stimulated early pilot studies with oral all-trans-retinoic acid and 13-cis-retinoic acid by Schumacher and Stüttgen (1971) and by our group in Cologne (unpublished), which basically revealed a definite clinical response. However, the risk of side-effects has been considered too high by us, compared with other effective drugs in acne, for example tetracyclines. Also, the teratogenic properties of retinoids in animal experiments require contraceptive measures in humans, particularly in young individuals, and these may themselves influence acne. In recent years retinoid-induced suppression of sebaceous gland activity and marked changes of human serum and surface lipid composition have been reported (Strauss *et al.*, 1978; Strauss *et al.*, 1980), raising new hopes for an adequate oral treatment. Indeed, patients with the cystic or conglobate type of lesions experienced complete clearing of their disease after 4 months treatment with 13-cis-retinoic acid (2·0 mg/kg bodyweight daily) and prolonged remissions have been seen after therapy (Peck *et al.*, 1978; Peck *et al.*, 1979). It is not yet quite clear whether other retinoids exert similar effects on the sebaceous gland; it appears that the aromatic retinoid Ro 10–9359 is less effective as an anti-acne drug.

It should be noted that, in contrast to psoriasis and other genodermatoses, acne is mostly a temporary episode in life, to be treated by local management only, with few exceptions. We do not feel that oral retinoids are appropriate drugs for routine use. It seems reasonable, therefore, to search for a topical new synthetic retinoid which does not result in local irritation, as all-trans-retinoic acid does, accompanied by sufficient transepidermal absorption to bring about the desired progress. Up to now topical treatment with the synthetic aromatic ethylamide (Ro 11–1430) has not been found curative in acne (Handojo, 1977; Christiansen, Holm & Reymann, 1977).

Lichen planus

Lichen planus, particularly its mucosal erosive type, seems to respond well to oral treatment with aromatic retinoid and with the 13-cis-retinoic acid stereoisomer (Schuppli, 1978; Scheiber & Plewig, 1978; Ebner, 1979). Similar results in lichen planus and lichen verrucosus were reported earlier by Günther (1975b). The disseminated type has also revealed marked improvement (Ebner, 1979); however, the disease seems to relapse after reduction of the dose or premature discontinuation of oral therapy. Medication should last for at least 2–3 months in low dosage (35–50 mg/d). At present it seems that oral retinoids are an effective alternative drug for lichen planus in cases with mucosal involvement or in patients with contraindications for oral corticoids, such as diabetes, osteoporosis, tuberculosis, etc.

Other conditions

Finally, a number of miscellaneous other skin conditions have been reported to improve markedly after oral therapy with aromatic retinoid: verrucous epidermal naevus (Happle, Kastrup & Macher, 1977), subcorneal pustular dermatosis of Sneddon-Wilkinson (Folkers & Tafelkruger, 1978), Netherton's syndrome (Duperrat *et al.*, 1977), epidermolytic hereditary palmoplantar keratoderma (Fritsch, Honigsmann & Jaschke, 1978), plantar warts (Rüst & Rufli, 1979), superficial actinic type of porokeratosis (Kariniemi, Stubb & Lassus, 1980), and disseminated porokeratosis of Mibelli (Bundino & Zina, 1980).

So far it seems that two oral retinoids may have considerable chances to become established as routine therapy in the next few years: (a) the aromatic retinoid Ro 10-9359 for a broad spectrum dermatotherapy, particularly in psoriasis, lichen planus, keratotic genodermatoses in general (including Darier's disease), and in precancerous lesions of the oral mucosa, and (b) the 13-cis-retinoic acid particularly for severe nodulocystic acne.

MODE OF ACTION

According to our present experience the aromatic retinoid Ro 10-9359 can obviously be used with favourable results in a large number of skin diseases at doses between 25 and 75 mg/day, its side-effects being rather rare and tolerable at this dosage. It is effective in all types of hyperkeratotic conditions with ortho- and parakeratosis, without being specific for any one disease, since it generally normalizes excessive keratinization processes.

By light and electron microscopy the drug seems to decrease the number and extent of keratin precursors in the Malpighian layer, such as tonofilaments and tonofibrils; it increases the ability of keratinocytes to synthesize mucous-like material, also increasing their size due to intracellular oedema. The desmosomes become smaller and less well developed, microvilli of the cell surface appear, and the stratum corneum loses its superficial layers and becomes thinner. These effects were clearly demonstrated in psoriatic skin under therapeutic conditions (Orfanos & Runne, 1978) and in guinea-pig epidermis one week after high dosage (Tsambaos, Mahrle & Orfanos, 1980). It is interesting to note that after 3 weeks of retinoid administration normal guinea-pig epidermis becomes acanthotic and hypergranulosis occurs. In other words, normal keratinocytes adapt themselves to the drug and restore gradually the retinoid-induced changes. These changes may differ according to site and possibly to the phase of the cell cycle or the number of available retinoid receptors in the tissue. Also in psoriasis the stratum granulosum, if lacking, reappears and may become hypergranulotic.

By autoradiographic studies it was shown that systemic treatment with aromatic retinoid in psoriasis reduces the increased [^{3}H]thymidine labelling index and shortens the prolonged DNA synthesis time after 3 weeks (Dierlich *et al.*, 1979). Changes of the altered epidermal cell kinetics towards normal were found in both involved and uninvolved skin in psoriasis.

These observations indicate that the aromatic retinoid Ro 10-9359 has a profound effect on proliferation and differentiation of keratinocytes and may thus influence skin diseases with epidermal involvement.

Additionally to this, recent observations in our department in Berlin suggest that the aromatic retinoid Ro 10-9359 has an immunomodulatory effect on dermal cell components: Under oral retinoid treatment atypical lymphocytes resembling Sézary cells, stimulated macrophages and dermal Langerhans cells appear in the dermal cell infiltrate in psoriasis (Tsambaos & Orfanos, 1980). Also, the aromatic retinoid and the free aromatic acid Ro 10-1670 as its main metabolite inhibit *in vitro* the mitogenic stimulation of PHA, ConA and pokeweed mitogen on human lymphocytes from normal individuals and patients with psoriasis (Bauer & Orfanos, submitted for publication). These findings indicate that the aromatic retinoid has a marked effect on both epidermal and dermal cell components.

We believe that oral retinoids act primarily on epithelial tissues but also have a distinct effect on monocytes and lymphocytes, modulating the cellular immune responses in mesenchymal tissue. It cannot be ruled out that this effect may have a major significance in the efficacy of oral retinoids as dermatotherapeutic drugs.

SIDE EFFECTS

No serious complications have been described up to now after oral administration of retinoids in therapeutic dosage over several months or years, in contrast to vitamin A (Fleischmann, *et al.*, 1977; Kistler *et al.*, 1977). The side-effects of the aromatic retinoid Ro 10-9359 grossly correspond to those of the natural metabolite all-trans-retinoic acid: cheilitis and hair loss are most common, seen in around 70% and 30% of cases respectively. Dryness of mucosal epithelia, thinning of skin, palmoplantar scaling, nasal or petechial bleeding, paronychia and vomiting may rarely occur. With few exceptions all side-effects are tolerable; they are also dose-dependent and fully reversible after discontinuing treatment or reducing the dose. Their appearance is clearly predictable, according to the dose and the duration of therapy, and independent of the underlying disease (Orfanos & Goerz, 1978).

Hair loss occurs, as a rule, 3–8 weeks after onset of oral medication with 50–75 mg/d, i.e. after a minimal total dose of 2·0 g oral retinoid. It ceases after 6–8 weeks if treatment is discontinued. Hair root evaluation showed a telogen effluvium in 70% of cases, whereas in 22% of cases telogen and dystrophic anagen roots were found (Mahrle *et al.*, 1979).

Computer evaluation of laboratory parameters in a large number of patients treated with Ro 10-9359 did not reveal any statistically significant alterations, although moderate elevations of SGOT and/or SGPT were recorded in some cases, indicating an individual sensitivity to retinoids, of unknown aetiology (Orfanos *et al.*, 1979a). The data revealed statistical trends towards slight elevation of the total serum bilirubin level and of the number of the peripheral blood monocytes.

Elevation of serum lipids was first described after oral administration of retinoic acid ethylamide by Runne *et al.* (1973). Similar changes have been recorded by several investigators during the last few years after oral treatment of acne with 13-cis-retinoic acid. Also the aromatic retinoid seems to cause elevation of triglycerides in some cases during the first weeks of oral treatment, particularly in those patients in whom the lipid values were high, but still within normal levels, before treatment. The lipid values in the long-term treatment are presently under careful investigation.

In spite of the lack of serious side-effects in the short term, careful consideration has to be given to the fact that the drug or its main metabolite may accumulate in the body over a longer period of time; necessarily, pregnancy has to be avoided during and 3–6 months after treatment, due to the drugs' embryotoxic and teratogenic properties. Changes in the gonadal epithelia and function have not been observed up to now; however, only a few investigations of sperm counts have been conducted before and after therapy. In animal experiments distinct changes occur after high dosage (25 mg/kg/d (Tsambaos *et al.*, 1980)), but they seem to be reversible after discontinuation of treatment like all other side-effects of oral retinoids.

Summarizing these data it appears to us that for the first time in the history of Dermatology a new group of drugs exerts its main pharmacological action almost exclusively on the skin and its appendages. In contrast to other compounds, such as antibiotics, corticosteroids and cytostatics, which were introduced into dermatological treatment in the late stages of their development, dermatologists and skin research laboratories are being considerably involved in the early experimental investigation on oral retinoids, and in systematic phase two clinical trials in view of their prospective therapeutic use. Their observations may also prove useful in related disorders such as the management of epithelial tumours: inhibitory activity of vitamin A acid and various retinoids in mammary carcinogenesis (Moon *et al.*, 1977), *in vitro* growth of malignant melanoma (Lotan *et al.*, 1978), precancerous oral lesions in humans (Koch, 1978) and human bronchogenic carcinoma *in vivo* and *in vitro* (Kistler & Peter, 1979) have been reported. Some of these tumours contain considerable amounts of retinoic acid-binding protein (Sani & Titus, 1977).

Thus, oral retinoids may also contribute to elucidating unsolved questions in future cancer research, since they obviously prevent experimental carcinogenesis. Their clinical application as an antitumour agent in oncology is now a subject for further and thorough investigation.

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Therapeutic evaluation of the oral retinoid Ro 10-9359 in several non-psoriatic dermatoses

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SUMMARY

Forty-five patients suffering from various genodermatoses and erythematous, scaling, non-psoriatic dermatoses were treated orally with the aromatic derivative of retinoic acid, Ro 10-9359 (Tigason).

In the genodermatoses the best results were obtained in ichthyosis, keratodermias and Darier's disease (95·6% good to excellent). Among the erythematous scaling diseases, treatment was effective in lichen planus, parapsoriasis and pityriasis rubra pilaris (53·3% good to excellent results).

In comparison with therapies previously employed Ro 10-9359 was more effective. No serious side-effects were noted.

Dermatological therapy in general, and the treatment of many disorders marked by hyper- and dyskeratosis in particular, have been improved lately by the use of retinoic acid and its analogues, the retinoids.

Among this group of drugs, the aromatic derivative Ro 10-9359, ethyl-all-trans-9-(4-methoxy-2, 3, 6-trimethylphenyl)-3, 7-dimethyl-2, 4, 6, 8 nonatetraenoate, has proved to be especially active in the treatment of psoriasis, whatever its variety or extent (Viglioglia & Barclay, 1978). In addition, the preparation has been shown to be effective in several non-psoriatic disorders, some of them resistant to any other treatment (Viglioglia & Traballi, 1979). The present paper reports the effects of the drug on a group of dermatoses, basically erythematous scaling non-psoriatic disorders and genodermatoses.

MATERIALS AND METHODS

The study has been carried out on forty-five patients who received the aromatic derivative orally, evaluating a 3 months' period of treatment. There were eighteen male patients (40%) and twenty-seven female (60%), with a mean age of 34·4 ± 18 years (range 3–76 years). The average weight and height were 66·1 ± 16 kg and 160·5 ± 15 cm (range 20–95 kg and 100–180 cm). Treatment was initiated with 1 mg/kg/day and the dosage was reduced to 0·7 mg/kg during the second and third months. The diagnoses of the patients are shown in Table 1.

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TABLE 1. Results according to diagnosis

		Results				
Diagnosis	No. of cases	Excellent	Good	Moderate	Insufficient	None
Ichthyosis vulgaris	13	4	9	–	–	–
Lichen planus (several kinds)	11	2	3	5	–	1
Congenital keratodermias	4	1	2	–	1	–
Acquired keratodermias	3	–	3	–	–	–
Congenital ichthyosiform erythrodermias	2	1	1	–	–	–
Parapsoriasis en plaques	2	–	1	1	–	–
Comedo naevus	2	–	–	–	–	2
Epidermal naevus	2	–	1	–	–	1
Basal cell naevus syndrome	1	–	–	–	–	1
Florid oral papillomatosis	1	–	–	–	–	1
Darier's disease	1	–	1	–	–	–
Pityriasis rubra pilaris	1	–	1	–	–	–
Tuberous acne	1	–	–	–	–	1
Parapsoriasis guttata	1	–	1	–	–	–
Total	45	8	23	6	1	7
Percentage	100·0	68·9		13·3	2·2	15·6

To evaluate the action of the drug the following were observed: erythema, infiltration, scaling and hyperkeratosis, assigning a score of intensity: 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Efficacy was assessed by evaluating each of the signs during and at the end of the treatment time, when an overall judgement was made, according to conventional criteria—excellent: complete remission of all signs; good: 75% improvement of the signs; moderate: 50% improvement; insufficient: slight improvement; and none or unchanged.

RESULTS

Table 2 summarizes the changes in the clinical signs. The changes observed were statistically significant for all the four signs—erythema, infiltration, scaling, and hyperkeratosis ($P<0{\cdot}01$). Figure 1 shows the intensity of the clinical signs before and after 3 months of treatment, and Table 1 the therapeutic response in general and according to diagnosis. It can be seen that the results were from good to excellent in 68·9% of patients, moderate in 13·3%, insufficient in 2·2%, and unchanged in 15·6%.

The side-effects caused by the retinoids are well known. All our patients experienced some side-effects, but treatment did not have to be suspended in any of the cases. Dryness of lips occurred in 100% of the cases, thinness of skin in 93·3%, dryness of oral and/or nasal mucosa in 82·2%, scaling in 42·2%, pruritus in 17·8%, alopecia in 15·6%, and rhagades in 4·4%. Figure 2 shows in detail the evolution of the side-effects, their intensity and relation to the dose given.

TABLE 2. Intensity of clinical signs

Sign	Score of intensity*	Cases					Binomial test†
		Before	15 days	30 days	60 days	90 days	
	0	2	4	5	4	5	
Erythema	1	5	7	7	9	15	*P*<0·01
(*n*=30)	2	2	8	7	13	6	Statistically
	3	21	11	11	4	4	significant
	Sum of scores	72	56	54	47	39	
	0	0	6	5	3	4	
Infiltration	1	8	9	13	19	22	*P*<0·01
(*n*=29)	2	10	9	7	4	0	Statistically
	3	11	5	4	3	3	significant
	Sum of scores	61	42	39	36	31	
	0	2	10	8	8	11	
Scaling	1	8	14	19	24	26	*P*<0·01
(*n*=41)	2	18	11	10	5	1	Statistically
	3	13	6	4	4	3	significant
	Sum of scores	83	54	51	46	37	
	0	0	4	1	4	5	
Hyperkeratosis	1	5	10	16	9	14	*P*<0·01
(*n*=25)	2	6	7	3	8	2	Statistically
	3	14	4	5	5	4	significant
	Sum of scores	59	36	37	37	30	

*0=none; 1=mild; 2=moderate; 3=severe.
†Improvement observed after 90 days treatment.

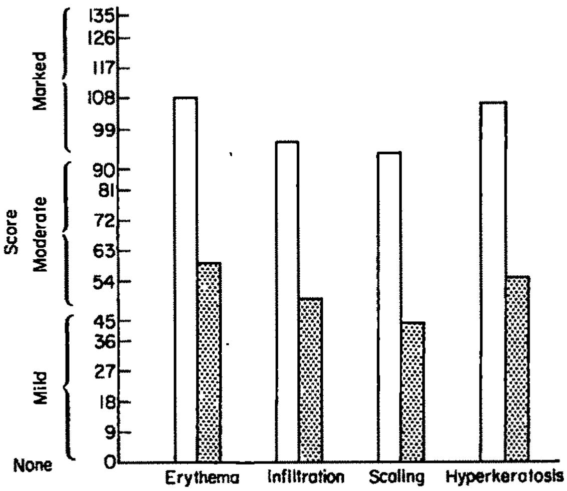

FIGURE 1. Evolution of clinical signs. (Open columns) before treatment; (stippled columns) after 90 days of treatment.

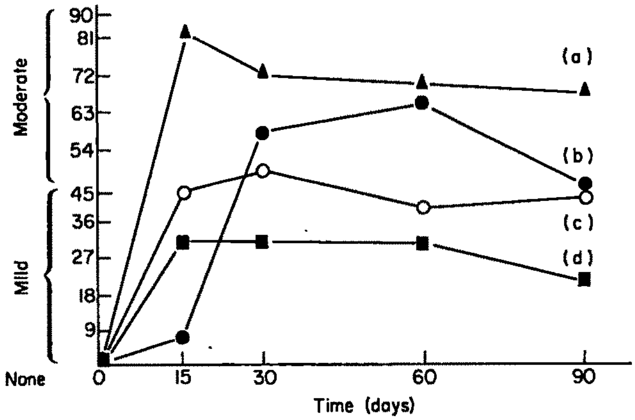

FIGURE 2. Evolution and intensity of side-effects and their relation to dose. Mean dose at 15 days=9 mg/kg/day; at 30 days= 0·8 mg/kg/day; at 60 days=0·68 mg/kg/day; at 90 days=0·71 mg/kg/day. (a) Dryness of lips; (b) alopecia; (c) thinness of skin; (d) dryness of nasal mucosa.

DISCUSSION

The retinoid Ro 10-9359 (all-trans-retinoic acid) is an analogue of retinoic acid and has the same tetraen chain. They differ in the ring moiety and the end group. Ro 10-9359 has a non-aromatic, partly unsaturated ring and a carboxy group. The 13-cis-retinoic acid differs from the all-trans-retinoic acid only in the position of the carboxy group.

After oral administration Ro 10-9359 reaches its maximum concentration in plasma of 600 ng/ml after 4 h (Hänni, 1978). In plasma, the ester group is enzymatically hydrolysed and the compound is transformed into the corresponding acid, which is its major biologically active metabolite. Within the first 8 days after oral administration of tritiated Ro 10-9359 (2 mg/kg) to humans, excretion in the urine accounted for 12% of the dose. In the same period the faecal excretion was 75%, mostly through biliary excretion. Experiments in rats revealed that the concentration of the compound in the liver is the largest in comparison with other tissues and reaches 16% of the dose given.

Oral retinoic acid has been beneficial in patients with both classical and bullous congenital ichthyosiform erythrodermia (Eriksen & Cormane, 1975). Favourable results have also been obtained in patients with lamellar ichthyosis, ichthyosis vulgaris, Darier's disease, pityriasis rubra pilaris, keratosis palmaris et plantaris and cystic acne. In two patients with the basal cell naevus syndrome complete regression, both clinically and histologically, was observed in 10% of the tumours (Peck *et al.*, 1978); a complete cure of a systematized verrucous epidermal naevus was also reported (Happle, Kastrup & Macher, 1977). Retinoic acid has also been reported as useful treatment for oral lichen planus (Günther, 1973a, b).

A recent multicentre study (Orfanos *et al.*, 1979) of 235 patients with psoriasis treated with 50–75 mg/day of Ro 10-9359 for several weeks or months (average 118·5±75·5 days) revealed a trend to elevation of the total serum bilirubin level, increasing number of blood monocytes and in a few cases the drug had an effect on the GPT and GOT levels. The reasons for this individual susceptibility to the drug remain unknown.

The mechanism of action of synthetic retinoids remains entirely unknown, but may be related to the known ability of vitamin A to affect glycoprotein synthesis and epithelial differentiation. In our study, the whole group of ichthyoses, including thirteen patients with ichthyosis vulgaris and two with congenital ichthyosiform erythrodermias, has undoubtedly been the most improved by the medication. Once the scaling, dryness and feeling of tightness disappeared, the skin became gradually more flexible, soft and smooth, and some of the hyperkeratosis disappeared too. The improvement obtained was undoubtedly superior to that obtained by the various therapies employed to date.

The same applies to the results observed in Darier's disease.

Three of the congenital keratodermias were of the Thost-Unna type and one of the intermediate form, also known as Greiter's syndrome. In the former, the results were satisfactory; the latter responded insufficiently. For acquired keratodermias, the therapy achieved obvious improvement, but the results in some naevoid abnormalities (comedo naevus, epidermal naevus, basal cell naevus), were variable or insufficient. Of the erythematous scaling non-psoriatic dermatoses, the largest group was that of eleven patients with lichen planus. Satisfactory results were obtained in half of the cases. Unlike some European authors (Schuppli, 1978), we had poor results in bullous and erosive oral lichen planus.

As to the remaining erythematous scaling diseases included in the study (parapsoriasis guttata; parapsoriasis en plaques; pityriasis rubra pilaris) the retinoid was generally beneficial.

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Pigment anomalies of the skin in the human chimaera: their relation to systematized naevi

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SUMMARY

Double fertilization of the egg may lead to a chimaeric state, in which the cell composition of the individual derives from two zygotes. Some special types of naevoid hyperpigmentation may result Two new cases are added to the three published cases of chimaerism with skin manifestations.

Of all the reasons for a disturbance in skin colour, the rarest is perhaps that in which the individual is formed from more than one zygote. These are the true chimaeras, in whom differing sets of chromosomes are presumed to result from a dual fertilization, provided that other sources of cell mixture are unlikely. At the blastocyst stage in embryonic life, in the mouse at least, there are possibly only three cells which actually go to form the body of an embryo (Markert & Petters, 1978). If these three cells are derived from two different zygotes, the starting proportions of the mixture will obviously lie at two-thirds to one-third.

When two sperms fertilize an ovum which contains an extra nucleus (e.g. a polar body) a chimaeric state results. It will not be suspected if the embryonic body happens to stem exclusively from one of the two available cell lines. However, if the body should contain cells with differing sets of chromosomes, this mixture of cells may eventually be revealed through the individual possessing a mixture of two kinds of blood or by intersexuality, should there happen to be an XX/XY mosaic of the sex chromosomes. An XX/XX mosaic will not disturb sexuality. Such spontaneous cell mixtures in chimaeras may also cause the skin to manifest two shades of colour, though not necessarily so (Race & Sanger, 1975).

Even if the original cell mixture, existing in a state of mutual tolerance in the embryo, could have been expressed as a simple mathematical proportion, cellular movements, selection and change with space and time during development are bound to disturb the initial ratio (West & McLaren, 1976; Stephens, McIvor & Wagner, 1977; Falconer & Avery, 1978).

Known zygote mixtures also affect the phenotype in ways that may be attributed to the cells of neither parent. When a healthy embryo is fused with an abnormal or lethal one at an early stage, the cells of the poor lines may be 'rescued' by the normal ones (Bennett, 1978; Le Stevens, 1978).

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The effects of a cell mixture may be imitated in the female by partial or total inactivation of one or other X chromosome, when the phenotypes are conspicuously dissimilar. For instance, should one of these X chromosomes carry a translocated gene for albinism, patterns of pigmentation arise which look remarkably like certain human chimaera skin patterns. Thus, in the so-called Cattanach translocation in mice, a patchwork of alternating dark and light areas results. They meet sharply in the ventral midline, and are clearly mismatched with one another (Fig. 1F). Similarly in the brindled mouse, despite smaller colour differences in the transverse barring, there may also be a gross asymmetry in colour development on either side of the midline (Fig. 1E). These findings and their interpretation are accessible in a valuable paper by Grüneberg (1969).

Grüneberg's views are in remarkable harmony with Thom's (1975) later concept of morphogenetic stability, arising by 'catastrophe' from a foregoing unstable state. The stable picture comes about by means of a threshold phenomenon, based upon the group behaviour of cells. It is preceded by a phase of instability due to combined inhomogeneities between genes, chromosomes and cells in a tissue. It is a harder concept to grasp than the belief in a simple pathway to pattern production. The simple view suggests a straightforward onward movement from gene-to-cell-to-tissue-to-pattern. Although this simple pathway provisionally accounts for certain animal colour patterns, it suffers from being generally unworkable, as Grüneberg cogently argued, and as observers of other mammals have long had reason to suspect.

Human chimaeras with patchy skin colour are now five in number—three are described in existing publications, to which we add two. One of our cases was reported earlier (Moores, 1973) but her skin was not fully described at that time. This lack has now been remedied. The other chimaera, which was discovered later, has not been reported before.

SKIN FEATURES IN THE REPORTED CASES OF HUMAN CHIMAERAS

Zuelzer, Beattie & Reisman (1964)

An 18-year-old 'male' of mixed Negro, Caucasian and Amerindian descent showed topographical differences in skin pigmentation. He was described as having skin of a pale milky coffee colour, some 10% of which was darker in certain sharply outlined areas. The areas consisted of irregular pigmented patches on the face, and darker wedges and bands abutting on the anterior and posterior midlines of the trunk. Figures 1B and C show the pigmentation pattern as it appears in the published pictures.

A prospective blood donor, this individual was found to have both group A and group B cells, and a slight gynaecomastia which was the sole clinical sign of intersexuality. Fibroblast cultures from the normal pale skin areas yielded XY cells only. The dark skin gave rise to more slowly growing fibroblasts, yielding XY and XX metaphases in a roughly 10:1 ratio. Tetraploid cells were common throughout.

Corey et al. (1967)

A white 'male' child was born with a bifid scrotum, extreme hypospadias, one ovary and one testis, although male characteristics predominated. The skin showed some general mottling, but the anterior abdominal wall on either side of the midline exhibited a darkening in roughly reciprocal quadrants. These darker areas tanned readily (Fig. 1A). The fibroblasts of the skin in two diagonally placed pale areas yielded 94% XX and 6% XY cells, while the corresponding dark zones yielded 60% XX and 40% XY, and 68% XX and 32% XY cells respectively.

Fitzgerald, Donald & Kirk (1979)

A white 16-year-old 'male' had hypospadias, gynaecomastia, one ovary and one testis. There were

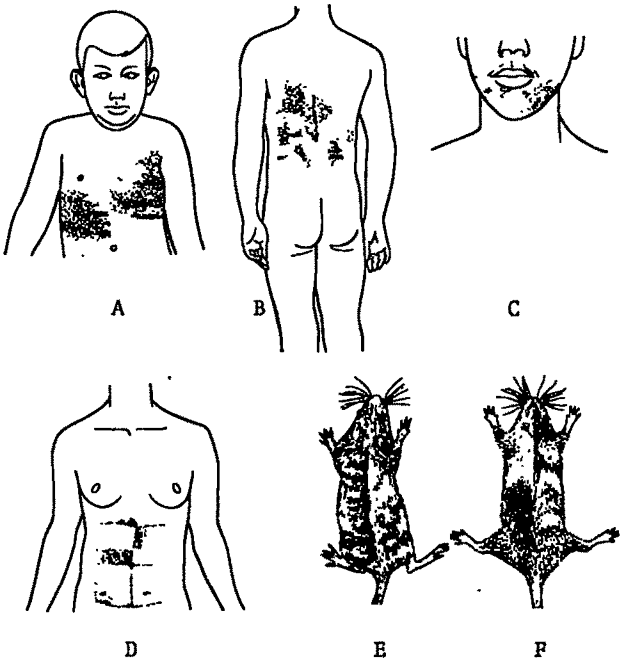

FIGURE 1. (A) Skin pigment mosaicism of the abdominal area. Redrawn from Corey *et al.* (1967). (B) Irregular localized pigmentation of the torso. Redrawn from Zuelzer *et al.* (1964). (C) Patchy pigmentation of the face. Redrawn from Zuelzer *et al.* (1964). (D) Segmental distribution of skin pigmentation of the trunk and gynaecomastia. Adapted and redrawn from Fitzgerald *et al.* (1979). The lower abdomen shows what we presume to be the two biopsy sites, taken from skin areas with relatively little colour contrast. (E) Brindled ($Mo^{br}/+$) female mouse aged 47 days, with dorsal midline effect. Dark and light areas meet sharply along the midline. Redrawn from Grüneberg (1969) (F) Flecked female mouse 61 days old with Cattanach's translocation. Crossed midline effects on the venter in the absence of similar dorsal effects. Redrawn from Grüneberg (1969).

segmented areas of darkened skin on either side of the abdominal midline with rectangular flag-like outlines. The patches suggested a checkerboard (Fig. 1D). Some horizontal dark lines and a marked linea nigra were also present. Fibroblasts from dark and light areas both yielded XX metaphases only, but a laparotomy incision for pelvic exploration yielded XX and XY fibroblasts in a 2:1 ratio. One presumes that the laparotomy sample was obtained in the midline from the region of the dark linea nigra.

CASES OF CHIMAERISM FROM NATAL

In more than a million routine blood tests at the Natal Blood Transfusion Service, only two samples were encountered suggesting a true chimaerism. Both were from 46 XX chimaeras, and both showed some remarkable skin features.

Case 1 (Moores, 1973)

This Indian woman was re-examined in 1979 to review the skin anomalies which were merely mentioned in the publication of 1973. On this more recent occasion the earlier blood findings were

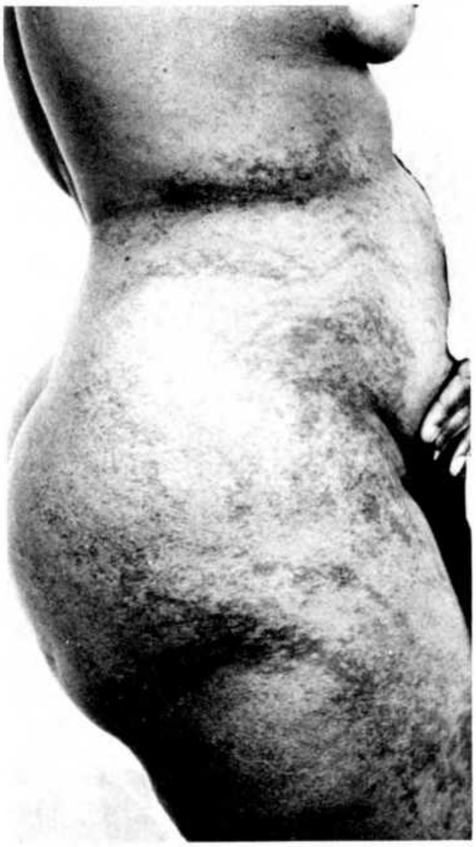

FIGURE 2. Case 1. Chimaera. Mottled, reticulate and striate pigmentation.

also confirmed. They are shown, together with new red cell enzyme results in Table 1. For other details, the original publication should be consulted.

In her case, widespread areas of pigment increase occurred all over the skin, arranged principally in striae. The areas were much more marked on the right half of the body, and were intensified where the striae ran together on the forearm or lower abdomen, and at sites of friction or sun exposure. These darker areas could be analysed into four interrelated patterns or grades of pigmentation, the mildest alteration being (i) a fine speckling or mottling, blending into (ii) a coarser reticulation consolidating into (iii) fine streaks or striae thickening into bands, and by fusion of bands into (iv) block-like dark masses.

The pigmented striae were to be found at or near the midline, down the length of the limbs and encircling the trunk. Their manner of arrangement recalled the pattern of epidermal naevi disposed along the so-called naevus lines of Blaschko. A number of these features are illustrated in Figs 2–4.

No histological examination was made.

Case 2

A healthy Black Zulu woman aged 30 donated a unit of her blood to the Natal Blood Transfusion Service. Though apparently of group O, her serum contained a weak anti-A_1 allo-antibody only.

TABLE 1. Red cell groups, red cell enzymes, leukocyte groups, and fibroblast cultures in two chimaeras from Natal

	Case 1		Case 2	
Red cell groups	55%	45%	99%	1%
ABO	B	A_1B	O	A_2B
MNSs	ss	Ss		
Rhesus	Rh_1rh	rh	No other differences were detected probably because of the small size of the minor cell population and the remarkable similarity of the parent's blood types	
Duffy	Fy (a+b−)	Fy (a+b+)		
Red cell enzymes				
Acid phosphatase (AP)	AB	A	AB	AR
Glutamyl pyruvate transaminase (GPT)	2	2–1		
Adenylate Kinase (AK)	1	2–1		
Esterase D (EsD)	2	2–1		
Peptidase A			8–2	1–1
Leukocyte Groups				
1st locus (LA)	HL-A1, W28	HL-A10		
2nd locus (Four)	HL-A8, W5	HL-A13±		
Fibroblast cultures	Not performed		No cell or chromosome abnormalities inside or outside the pigmented zones	
Chromosomes	All normal, 46XX		All normal, 46XX	

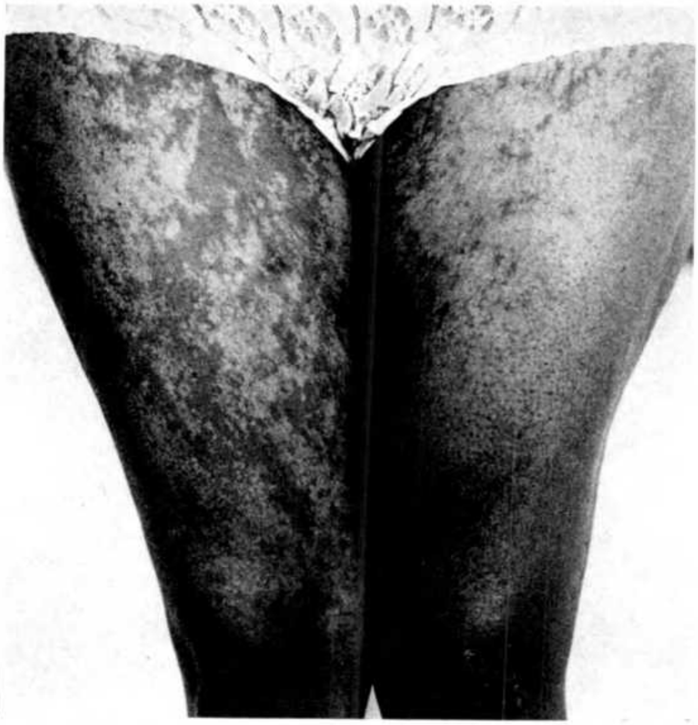

FIGURE 3. Case 1. Chimaera. Expansion of pigment network and bands into darker, block-like masses and wedges.

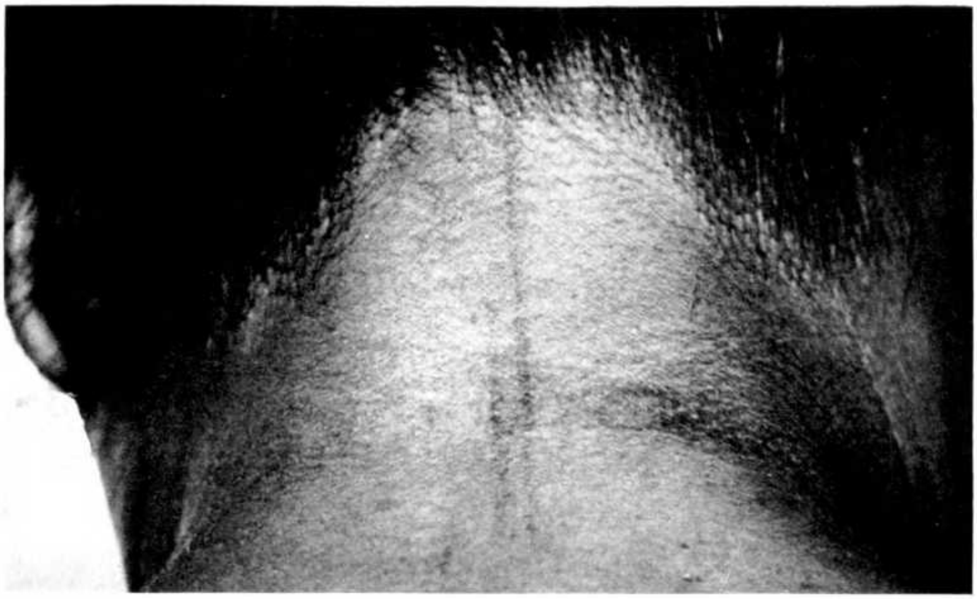

FIGURE 4. Case 1. Chimaera. Vertical arranged parallel pigment lines in nape of the neck. Frictional pigmentation, horizontally disposed from a necklace, more prominent on the right than left.

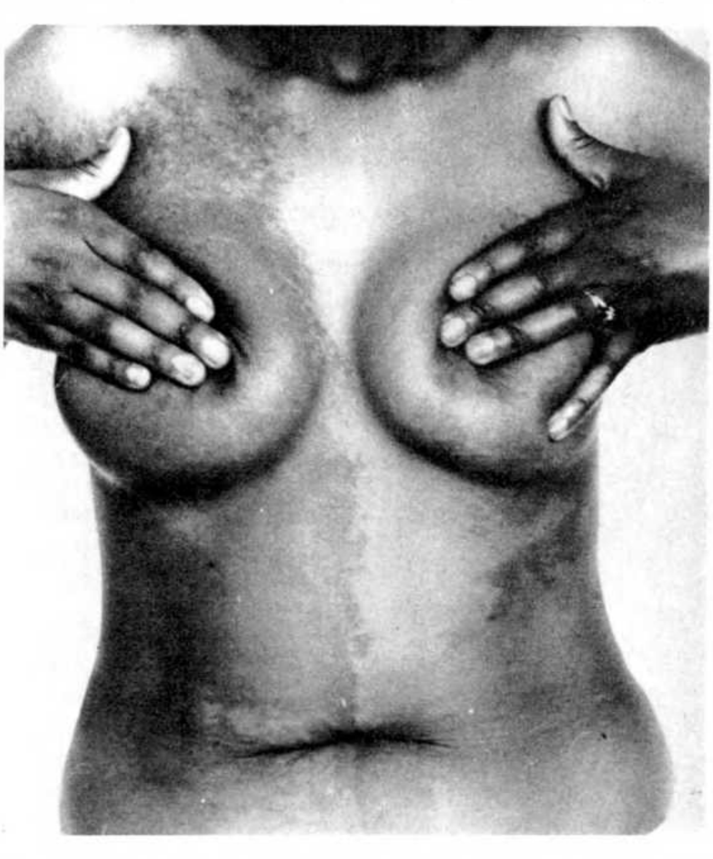

FIGURE 5. Case 2. Chimaera. Increased pigmentation on the right is confined between the midline, the 'centaur' line at the waist below, and the first intercostal space above. On the left a few bands of pigment reach the lateral edge of the rectus sheath.

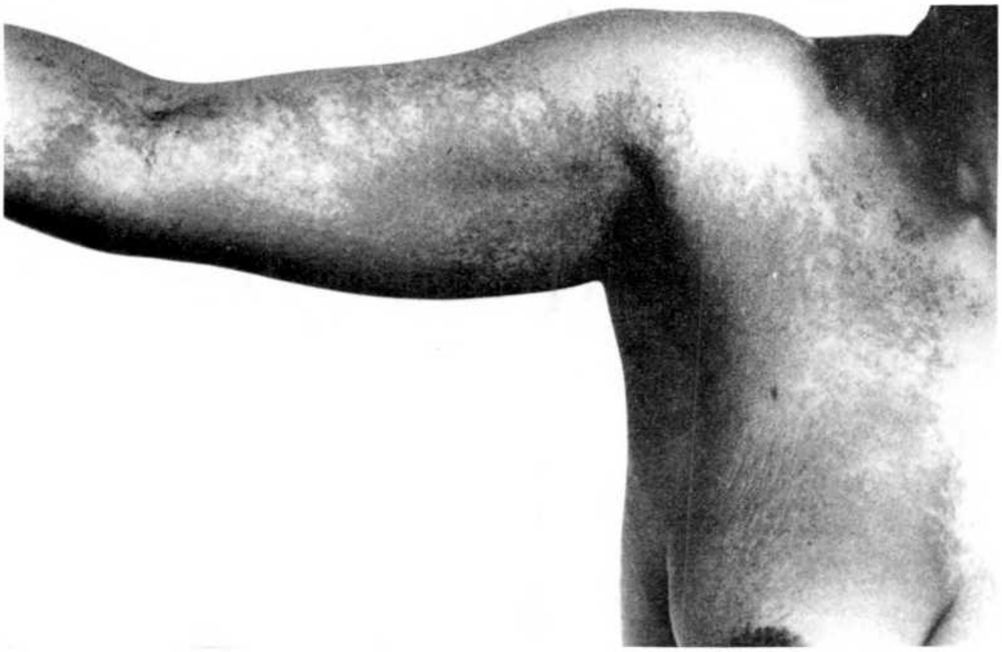

FIGURE 6. Case 2. Chimaera. Spray-like and granular pigmentation forming café-au-lait like patches, e.g. just below the elbow. Posterior half of the arm circumference mainly affected, medial to the area where Voigt's line would lie.

Further studies showed that her blood contained 99% group O and 1% A_2B red cells. These and other findings are listed in Table 1.

She was married, with two normal male children. Her pale brown skin was marked by large patches of unusually dark brown pigment, which had evidently not changed since birth.

The patches of abnormally dark skin colour (Figs 5–7) were restricted to the trunk and backs of the arms. The upper and lower limits of this zone could be clearly marked out by horizontal lines. By abducting the arm to a right angle, the upper limit was seen to run horizontally from the 2nd thoracic spine behind and the 2nd rib in front out to the head of the ulna. The lower limit ran horizontally round the trunk at the level of the anterior superior iliac spine. This pigmented zone abutted roughly at right angles on the dorsal and ventral midlines, tending to make square-cut edges. The quota of pigmentation between these limits was very different on the two sides of the body. On the right side the whole region was packed with heavily pigmented areas of skin, while the left side showed only a mild and incomplete suggestion of the full picture.

In contrast to our previous case, this subject's pigmentation, on the right side at least, could be graded into (i) a paler, fine, spray-like pigmentation, with a fine granularity of 1 mm or less, present at the upper limit of the area. Further down, the area contained (ii) larger solid rounded patches of varying darkness, resembling the café-au-lait spots in neurofibromatosis, with a paler background interlaced between them. At the lower limit of the area the pigment was (iii) confluent and darker than elsewhere. On the left side the pigmentation did not reach the midline, either in front or behind, and was arranged anteriorly in solid fingers pointing towards the rectus sheath and posteriorly in finer mist-like patches extending over the left shoulderblade. Skin from pale and dark areas of the

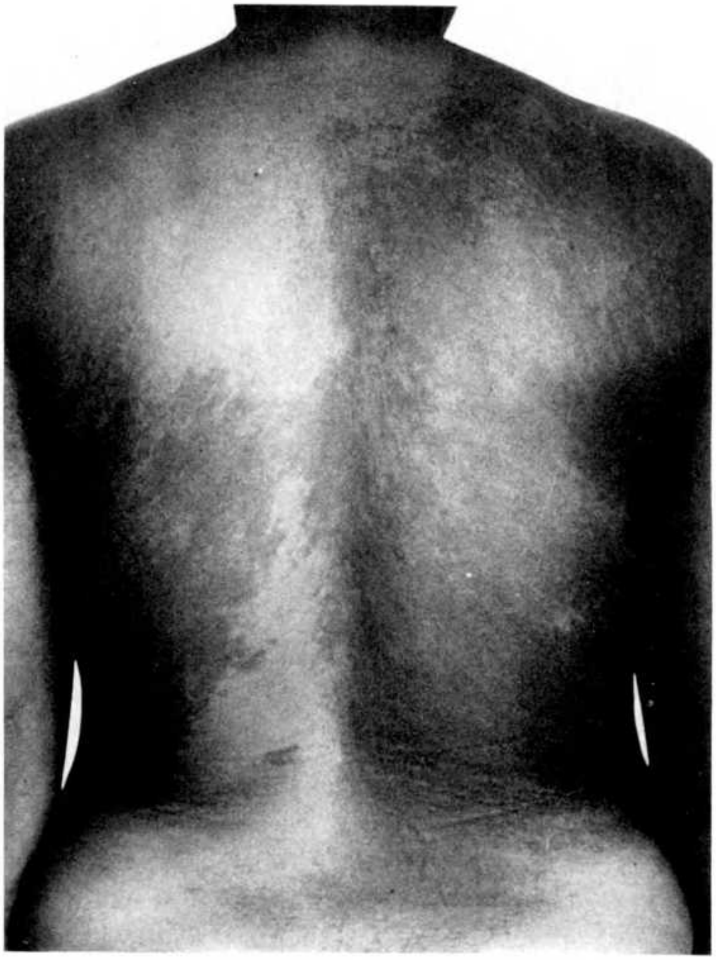

FIGURE 7. Case 2. Chimaera. Pigment limits on the right side of the back are seen at the level of T_2, the midline and horizontally in the lumbar region. On the left the pigment is finer and is mostly situated well away from the midline.

upper back were compared histologically with H & E and silver stains. Both were normal in structure and cell composition, but possibly in the skin from the darker area the melanocytes were plumper, producing more pigment, loading the basal and suprabasal cells of the epidermis more heavily. Nevertheless, epidermal pigmentation was uneven in both specimens.

Under the electron microscope some slight differences were seen between the epidermal cells of the two pieces of skin. Looser textured nucleoli and smaller, conglomerate melanosomes were seen in the darker skin, though perhaps these lay within the range of normal.

DISCUSSION

It is impossible to guess what a human chimaera ought to look like, and so our two chimaeras naturally surprised us in several ways. Their skin pigmentation could be divided into two regions: (i) a major region normal in appearance and (ii) a minor region, darker than one might expect if the skin had belonged to 'the other twin'. Evidence published by others also suggests that the fibroblasts in chimaeras may be more mixed in the darker skin areas and more homogeneous for one or other of the two cell lines in the normal skin areas.

Three main types of pigment pattern are recognizable in chimaeras. (1) A flag-like rectangular pattern. This is seen also in generalised naevus spilus (Davis & Shaw, 1964) and the Cattanach translocation (Grüneberg, 1969). (2) A pattern of rounded units. This is analogous to the café-au-lait patches in Von Recklinghausen's disease. (3) A striate pattern. This imitates the systematized epidermal naevus.

The increased pigment is strikingly collected into large compartments often with straight edges and sometimes having a box-like shape. Checkerboard patterns and marked asymmetry are evident, almost indicating that increased pigment at one block-like site may actively prevent its occurrence in the opposite half of the body across the midline, or up and down on the same side.

It is not really clear why the darker skin regions in chimaeras have such clean and strict outlines, or how they interact with the paler reciprocal areas in and around them. Even the innocent-seeming dorsal and ventral midlines, the obscurer naevus lines of Blaschko or the more common pigment demarcation lines (Matzumoto, 1913; Selmanowitz & Krivo, 1975) in normal individuals have not been accounted for, although they often serve as barriers for normal and pathological processes, as they do in chimaeras as well. Another line which may usefully be added to this assembly of unexplained lines lies horizontally across the lower abdomen and was evidently a pigment barrier in our second chimaera. Interestingly, this line is also the cut-off point in cases of superior lipodystrophy. We have called it the 'centaur line'. In this position the human part of a centaur changes into an animal, just below the waist, according to mythical tradition.

It would be attractive if one could discover a unitary scheme to account for chimaeric, naevoid and genetic patterns among abnormalities in the skin. The rival possibilities of a stem cell, organ system, tissue layer or cell mixture have to be considered, and these need to blend satisfactorily with induction processes, threshold phenomena and action at a distance. Judging by the chimaeric state, it seems that the somatic mesoderm and the neural crest contributions to the connective tissue appear to have as good a claim as any to cause the stepwise demands for pigment induced by this cell mixture on the epidermis. In the complex situations we meet with in clinical dermatology, a basic scheme of melanoblast clone migration (Mintz, 1974) is less satisfactory than one in which the melanoblast is merely the servant of a far more elaborate process, the colour level then being induced in the epidermis by the composition of underlying connective tissue.

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Changes in proliferative activity as cells move along undulating basement membranes in stratified squamous epithelium

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SUMMARY

The filiform papillae of mouse tongue provide evidence for a flow of interphase basal cells along the basement membrane. This implies that they are aligned on that structure according to age.

In addition there is a decrease in proliferative potential as the basal cells age. This is probably due at least in part to a fall in growth fraction. Such a method of basal cell replacement may exist in other stratified squamous epithelia characterized by undulating basement membranes.

In stratified squamous epithelium containing morphologically recognizable units of proliferation (epidermal proliferative unit, EPU, Potten, 1974), e.g. mouse epidermis, there is a marked variation in proliferative capacity amongst the basal cells (see Hume & Potten, 1979a for examples). In this relatively flat epithelium a limited lateral movement takes place within the basal layer of each unit but the bulk of cellular movement is towards the surface, at right angles to the basement membrane.

Oral epithelia tend to have pronounced rete ridges interdigitating with corresponding connective tissue papillae, producing a markedly undulating basement membrane (a situation typical of many stratified epithelia). A commonly held view of this type of epithelium is that all basal cells proliferate and do so in a homogeneous manner. Perhaps as a consequence of these concepts it is assumed that basal cells are 'fixed' in position and do not migrate to any significant extent laterally but, as in epidermis, produce cells that migrate distally.

The filiform papilla of the anterior two-thirds of mouse tongue contains the oral equivalent of the epidermal EPU (Hume & Potten, 1976) and permits cell kinetic investigation of individual basal cell positions on the connective tissue papillae. We have been using this system as a model for investigation of cell kinetic parameters in stratified epithelium.

This report provides evidence that the basal cells lining connective tissue papillae in stratified squamous epithelium may flow along the basement membrane to a significant degree. This implies that the higher cell positions are derived from, and are dependent upon, lower cell positions, i.e.

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basal cells are arranged on the connective tissue papillae according to their age. It will also be shown that as cells move along the basement membrane their proliferative ability decreases.

MATERIALS AND METHODS

Labelling studies

(1) BDF-1 male mice 9–10 weeks old were injected with 50 μCi methyl-^{3}HTdR at 1200 hours. Groups of three mice were killed after 40 min, 24 h, 48 h and 72 h.

(2) DBA-2 male mice 6–7 weeks old were given six injections of 10 μCi methyl-^{3}HTdR (one every 4 h) beginning at 24.00 hours. Groups of three mice were killed at 40 min, 24 h, 36 h and 72 h.

Mitotic studies

BDF-1 males, 8–9 weeks old were killed in groups of five at 3 h intervals over a single 24-hour period.

Tissue processing

Whole tongues were fixed in Carnoy's fixative for 20 min before being processed for paraffin embedding. Sections 4 μm thick were cut in the longitudinal axis. In labelling study (1), autoradiographs were exposed for between 9 d (40 min sample) and 22 d (3 d sample). In labelling study (2) all autoradiographs were exposed for 70 d (the injection regime and autoradiograph exposures were selected as part of a different experiment). Slides were stained with Feulgen or haematoxylin and eosin stains. The basal cells beneath and associated with the anterior aspect of the filiform papillae were numbered according to their position on the connective tissue papillae, position 1 being adjacent to the boundary between the anterior aspect and the adjacent interpapillary area. In some cases the anterior aspect may consist of a column of maturing cells. Alternate sections were scored until fifty well oriented papilla profiles had been examined for each animal (see Hume & Potten, 1976 for further details of the structure and the method of scoring).

RESULTS

Circadian data

The circadian mitotic index (MI) curves for the anterior basal cells are shown at Fig. 1. These exhibit the same trends as the labelling index (LI) curves for both the anterior and posterior aspect basal cells (Fig. 2). As increasing cell positions on the connective tissue papillae are considered there is a decrease in the size of the MI and LI peaks. No labelling usually occurred above position 4 for the anterior, and position 5 for the posterior basal cells lining the front and rear, respectively, of the connective tissue core. There is a marked synchrony within and between different cell positions shown by the 55–65% LI that can result from a single injection and by the fact that all peaks occur at 03.00 hours (LI curves) and 06.00 hours (MI curves) irrespective of position.

Labelling studies

The LI data are shown in Tables 1 (labelling study (1)) and 2 (labelling study (2)).

Table 1 indicates that the majority of cells at positions 1 and 2 are unlabelled and higher positions are completely unlabelled 40 min after injection. Subsequently the labelled front moves upwards with time.

The data presented in Table 2 show that all anterior basal cells irrespective of position are labelled 1 d after the last of a series of closely spaced injections of ^{3}HTdR.

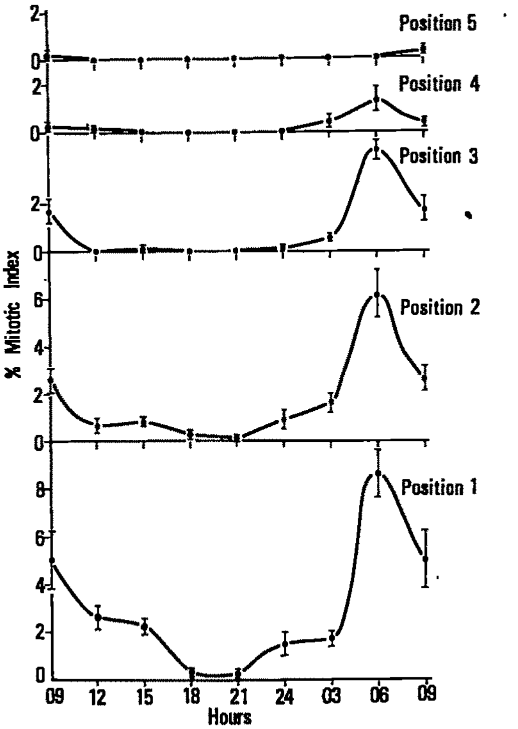

FIGURE 1. Circadian MI curves for individual basal cells of the anterior aspect of filiform papillae. Each point represents the mean ± s.e.mean of fifty papilla profiles from each of five animals.

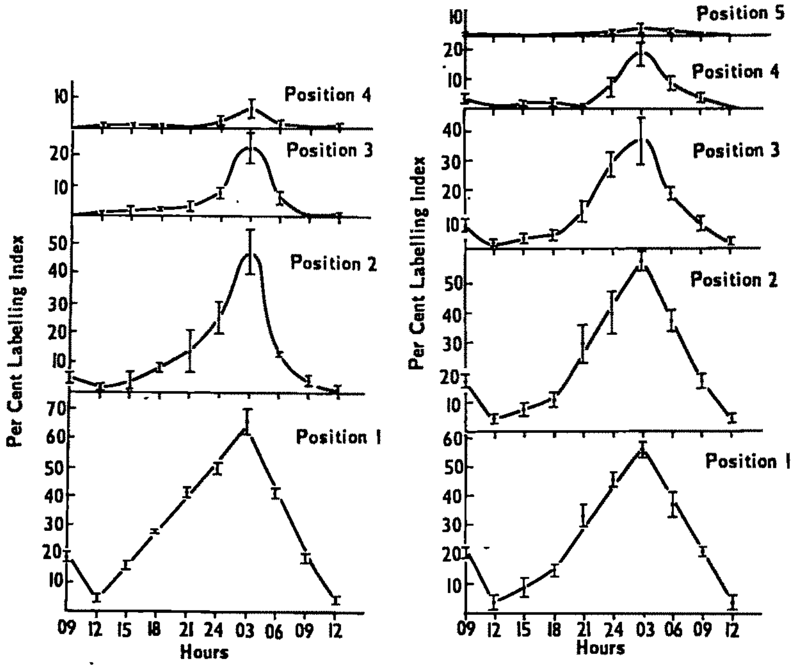

FIGURE 2. Circadian LI curves for anterior (left) and posterior (right) aspect basal cells of filiform papillae. Fifty papilla profiles from each of three BDF-1 females, 10–12 weeks old, were scored to give the mean ± s.e.mean for each time point.

TABLE 1. Percentage LI after 50 μCi ^{3}HTdR at 12.00 hours

	Anterior column			
	Time after ^{3}HTdR injection			
Basal cell position	40 min	1 d	2 d	3 d
5	0	0	63·7±7·7	95·0±3·0
4	0	5·7±0·3	83·0±1·0	92·0±0
3	0	32·4±2·3	90·0±0	94·0±2·0
2	3·3±0·7	36·3±5·2	86.0±2·0	95·0±1·0
1	5.3±0·7	34·4±2·9	79·0±9·0	83·0±1·0

Note: The LI at position 1 undergoes three doublings, i.e. increases seven-fold between 40 min and 1 d. During this time it is believed that many of the cells at position 1 divide only once (from circadian data, Potten *et al.* 1977; from delayed ^{3}HTdR labelling studies, Hume & Potten, 1979b; from unpublished PLM data). 14–16% of the increase is due to an average of one of the 6–7 cells at position 1 incorporating ^{3}HTdR into DNA 16 h after injection (delayed ^{3}HTdR labelling, Hume & Potten, 1979b). There is no evidence of delayed incorporation of ^{3}HTdR at other basal cell positions 18 h after injection, i.e. at 06.00 hours (Hume & Potten, 1979b). Further, the circadian data in Fig. 2 show that too few cells are in DNA synthesis between 06.00 hours and 12.00 hours (the day 1 point) for the cell flow data to be complicated by delayed ^{3}HTdR labelling at these higher positions.

TABLE 2. Percentage LI after six injections (one every 4 h) of 10 μCi ^{3}HTdR beginning at 24.00 hours

	Anterior column			
	Time after HTdR injection			
Basal cell position	40 min	1 d	1·5 d	2 d
5	15.3±2·4	99·3±0·7	100	100
4	48·7±5·8	99·3±0·7	100	100
3	84·7±2·9	100	100	100
2	100	99·3±0·7	100	100
1	100	100	99·3±0·7	86·7±2·9

Note. The fall in LI at position 1 on day 2 has been considered previously and may provide evidence for selective segregation of stem cell DNA (Potten *et al.*, 1978). The position 1 LI at day 3 (Table 1) is also consistent with this concept.

DISCUSSION

The anterior aspect or column represents a self-contained proliferative unit whose basal cells are responsible for all cell production, there being little if any entry of cells from adjacent columns or units (Hume & Potten, 1976). The data in Table 1 provide evidence for a significant flow of interphase basal cells along the basement membrane from low to high cell positions. The cells at positions 4 and 5 would seem to have little capacity for proliferation (as indicated by the circadian curves) yet they are almost completely labelled 1 d after the last of a series of injections (Table 2). The most likely explanation is that cells at higher positions are replaced from below by a flow of basal cells. The labelling data suggest that a similar flow occurs amongst the basal cells lining the rear of the connective tissue papilla.

Details of the suggested flow patterns are summarized in Fig. 3. As well as passing suprabasally into the columns from the highest cell position some labelled cells leave the basal layer from positions 1 and 2 at early times. This agrees with the finding of Cameron (1966).

The decreasing proliferative indices as cells age and move to higher basal cell positions can be achieved by (a) a lengthening of the cell cycle time (T_c) and/or (b) a decrease in growth fraction (GF). The ease with which 100% labelling of position 1 can be achieved with three closely spaced injections (Potten *et al.*, 1978) suggests that GF = 1 for this position in the anterior column. It is likely that GF does reduce with increasing position as the area under the MI and LI curves falls yet the synchrony as shown by the coincident timings of the peaks is still present. A *gradual* lengthening of T_c is unlikely therefore. Preliminary PLM data (unpublished) show little change between individual cell positions with regard to T_s estimates and the timing of the second peaks. This suggests that the fall in LI with increased cell position may be a reflection of a reducing growth fraction. In the posterior column a substantial number of cell positions exists above position 5. Usually these neither incorporate ^{3}HTdR nor divide. This finding combined with the change in morphology of these cells overlying the apex of the connective tissue papillae suggests that they are post-mitotic cells, i.e. the GF = 0.

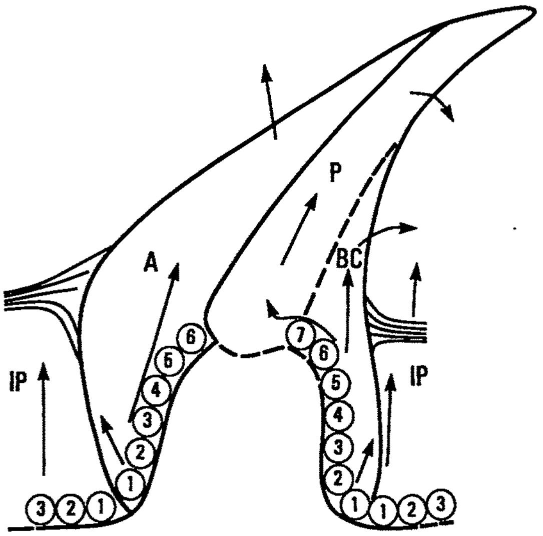

FIGURE 3. Diagrammatic representation of the structural organization of a filiform papilla. The method of basal cell numbering and the pattern of cell flow and desquamation are shown (A = anterior, P = posterior, BC = buttress column, IP = interpapillary. See Hume & Potten, 1976.)

If ^{3}HTdR is made available on a continuous basis (e.g. as a series of closely-spaced injections) all the basal cells overlying the apex of each connective tissue papilla become labelled after approximately 4 d (Fukuda *et al.*, 1978). We have found this complete labelling to occur 5–6 d after a series of only three closely spaced injections centred on the time of the LI peak. The transit data of Cameron (1966) following continuous availability of ^{3}HTdR in the drinking water are in broad agreement with our timings. The extra time required to completely label the posterior aspect basal cells is a reflection of the significantly greater number of them compared with the anterior column. It will, therefore, take longer to replace them by a flow of labelled cells. 'Transit' time measurements are approximate and depend on the initial position of the labelled front. Figure 2 shows that an injection of ^{3}HTdR at 03.00 hours produces a labelled front far in advance of that achieved by an injection at 12.00 hours.

A complicating factor is that after 3 d reutilization of ^{3}HTdR occurs from nuclei broken down in the stratum granulosum. This provides a continuous source of low-level label for several days (Hume, unpublished observation). Nevertheless the data suggest a cell flow even after this time. Fukuda *et al.* (1978) concluded that the delay in obtaining 100% labelling indicated that 67% of basal cells were in Go for periods of up to 100 h. The data presented here suggests that this delay is simply a consequence of the time taken for labelled basal cells to reach these positions.

The origin of the flow must be position 1. Evidence has already been presented that of the 6–7 cells at this position in the anterior column only one might be the stem cell if selective segregation of DNA is used as the criterion of 'stemness' (Potten *et al.*, 1978). It is not yet possible to state if some of the remaining position 1 cells are stem cells that lack this selective pattern of DNA segregation.

The concept that basal cells move along basement membranes and experience a gradual decrease in proliferative capacity as they do so is not dependent on the presence or absence of a superimposed columnar structure. Lateral migration along basement membranes clearly does occur in other situations, e.g. in intestinal crypts (Cairnie, Lamerton & Steel, 1965). Such a method of basal cell replacement may exist generally in those stratified squamous epithelia characterized by a flat surface and an undulating basement membrane. Further evidence for and consequences of such a model were considered by Hume & Potten (1979a).

ACKNOWLEDGMENTS

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The plasma membrane of Malpighian cells from pig epidermis: isolation and lipid and protein composition

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SUMMARY

A plasma membrane fraction from Malpighian cells has been isolated by differential and density gradient centrifugation of a pig epidermal homogenate. It was enriched in the marker enzymes 2-naphthylamidase, 5′-nucleotidase, phosphodiesterase I and acid phosphatase and depleted of NADH-ferricyanide reductase and cytochrome c oxidase. It had a protein to lipid ratio of 3:2 by weight. The protein composition was complex with compounds ranging from a molecular weight of 150,000 down to 13,000. Major components with molecular weights 120,000 to 90,000 were glycoproteins. Two other components had molecular weights of 39,000 (actin ?) and 24,000. There were minor components with molecular weights from 63,000 to 46,000. About 76% of the total lipid was present as phospholipid, which was enriched in sphingomyelin. Most of the neutral lipids were accounted for by cholesterol, triacylglycerols and fatty acids: very little glycosphingolipid was present. The preparation was probably derived from non-desmosomal areas of the plasma membrane of Malpighian cells, as desmosomes were not seen in the preparation.

The preparation of a plasma membrane fraction from full-thickness rat skin has been described (Lord, Ziboh & Warren, 1978) and we have reported the isolation of plasma membrane from pig granular cells (Gray, King & Yardley, 1978). As part of a continuing study of the changes that occur in the plasma membranes of keratinocytes as they differentiate into stratum corneum cells, we have isolated a plasma membrane fraction from pig Malpighian cells and determined its lipid and protein compoition.

MATERIALS AND METHODS

Substrates used for the biochemical assays were obtained from Sigma Chemical Co. All of the chemi-

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Dr G. M. Gray died in April 1980. An obituary will appear.

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cals were reagent grade. Uniformly [^{14}C] labelled adenosine monophosphate (AMP) was obtained from The Radiochemical Centre, Amersham. Sodium dodecyl sulphate was obtained from Serva Fine Biochemicals. Other reagents for electrophoresis were obtained from British Drug Houses and had been specifically purified for electrophoresis.

Pigs' ears were obtained from the abattoir within 2 h of death. Ears were cleaned with 5% (w/v) cetrimide and thoroughly washed with water. After removal of the hair with clippers, slices were taken from the skin with a Davies dermatome (Chas. F. Thackray Ltd, Park Street, Leeds) at setting No. 2 giving a nominal slice thickness of 0·3 mm. Histological examination showed that the slices contained all the epidermis with less than an equal amount of largely acellular epidermis. Portions of skin weighing about 2 g were shredded with scissors and transferred to glass tubes (25 mm diameter) with 10 ml ice-cold 0·25 mol/l sucrose-5 mM tris-HCl, pH 7·4. The tissue was homogenized with a Polytron homogenizer fitted with a PT20 saw-type generator for 10 s at speed setting 4 (16,000 r.p.m.). The homogenate was filtered through nylon bolting cloth with a hole size of 0·13 mm × 0·13 mm (Nybolt 10-132, obtained from John Staniar & Co., Sherborne Street, Manchester M3 1FD). The residue was re-homogenized in a further 10 ml of medium and filtered. The combined filtrates were further filtered through fine bolting cloth (hole size 0·02 mm × 0·02 mm).

Differential centrifugation

The filtered homogenate in 0·25 mol/l sucrose-5 mM tris-HCl (pH 7·4) was centrifuged at 2000 r.p.m. (700 gmax) for 10 min in glass tubes in a swing-out rotor to yield a crude nuclear fraction (fraction N). The supernatant was centrifuged at 17,000 r.p.m. (35,000 gmax) in the 8 × 50 ml rotor of a M.S.E. High Speed 18 centrifuge and the pellet washed once with 0·25 mol/l sucrose-5 mM tris-HCl (pH 7·4) to yield a crude mitochondrial fraction (fraction M): plasma membrane was subsequently isolated from this fraction by zonal centrifugation. The supernate and washings from the 35,000 gmax pellet were centrifuged at 40,000 r.p.m. (160,000 gmax) for 1 h in the 60 Ti rotor of a Beckman L2 65-B ultracentrifuge to yield a crude microsomal fraction (fraction P). The supernatant from the 160,000 gmax pellet was designated the soluble fraction (fraction S).

A portion of the filtered homogenate was also centrifuged at 40,000 r.p.m. for 1 h to yield a pellet consisting of epidermal homogenate freed of all soluble proteins. Subsequently, specific activities of enzymes in sub-cellular fractions were related to this material, rather than the original homogenate, in order to assess the enrichment of membrane-bound enzymes.

Zonal centrifugation

Gradients were formed in a Beckman Ti-14 zonal rotor spinning at 2000 r.p.m. in a Beckman L2 65-B ultracentrifuge. A step gradient was formed using 140 ml 0·5 mol/l sucrose, 160 ml 1·0 mol/l sucrose, 160 ml 1·5 mol/l sucrose and enough 2·5 mol/l sucrose to fill the rotor (approximately 200 ml). The solutions were sequentially pumped into the zonal rotor beginning with the 0·5 mol/l sucrose. Fraction M, obtained by differential centrifugation, in 20 ml 0·25 mol/l sucrose-5mM tris-HCl (pH 7·4) was added to the loaded rotor followed by an overlay of 20 ml water. After loading, the rotor was closed and brought to 45,000 r.p.m. for 1 h. The rotor was unloaded at 2000 r.p.m. by pumping 2·5 mol/l sucrose into the edge of the rotor and collecting the gradient from the centre. Thirty-three fractions of 20 ml were collected.

Membranes collected at 0·7 mol/l sucrose (fraction I), 1·25 mol/l sucrose (fraction II) and 1·9 mol/l sucrose (fraction III) were diluted with 5 mM tris-HCl (pH 7·4) to less than 0·5 mol/l sucrose and centrifuged at 40,000 r.p.m. (160,000 gmax) for 1 h in the 60 Ti rotor to obtain cell membrane pellets. Pellets were suspended in 0·25 mol/l sucrose-5mM tris-HCl (pH 7·4).

Assays

5′-Nucleotidase was measured using [^{14}C] 5′-AMP as a substrate. An inhibitor of 5′-nucleotidase, α, β-methylene adenosine diphosphate, was used and the enzyme activity was measured as the difference between total phosphatase activity and inhibitor-insensitive activity (Gentry & Olsson, 1975). 2-Naphthylamidase was measured by a modification (Gray, Tabiowo & Trotter, 1977) of the fluorimetric method of Lauter, Solyom & Trams (1972) using leucyl-2-naphthylamide as substrate. Phosphodiesterase I was measured by the method of Taylor, Williams & Crawford (1977) using thymidine 5′-monophospho-p-nitrophenyl ester as substrate. Acid-phosphatase was measured by incubating enzyme in 0·25 ml with 0·25 ml of p-nitrophenyl phosphate (2 mg/ml) in 0·1 mol/l acetate buffer (pH 5·0) at 37° for 60 min. At the end of this time, 0·025 mol/l sodium hydroxide (2·5 ml) was added and the absorbance was measured at 400 nm. Cytochrome c oxidase was measured by the method of Cooperstein & Lazarow (1951), and NADH-ferricyanide reductase was measured by the method of Strittmatter (1967). Protein was measured by the method of Lowry *et al.* (1951). Sucrose concentration was measured with an Abbé refractometer.

Extraction and analysis of lipids

The methods of isolation, identification and quantitative estimation of lipids have been described previously (Gray & Yardley, 1975a; Gray, 1976).

Electron microscopy

Subcellular fractions in less than 0·5 mol/l sucrose were centrifuged at 40,000 r.p.m. (160,000 gmax) for 1 h in the 60 Ti rotor, fixed and stained. The pellets were fixed in glutaraldehyde (2·5%) in 0·1 mol/l phosphate buffer, pH 7·4 at 4°C, for 30 min, rinsed with 0·1 mol/l phosphate buffer and allowed to stand in buffer at 4°C overnight. The buffer was decanted and the pellet was postfixed with phosphate-buffered osmic acid (1%) for 30 min, dehydrated through graded alcohol solutions, and embedded in Epon resin. Sections were cut and stained with saturated uranyl acetate in 50% (v/v) ethanol and Reynold's lead citrate for 10 min before examination in the electron microscope.

Solubilization and electrophoretic analysis of proteins

Subcellular fractions (5–10 mg protein/ml in 5 mM tris-HCl (pH 7·4), 0·25 mol/l sucrose) were incubated with 2% sodium dodecyl sulphate, 10 mM 2-mercaptoethanol at 100°C for 10 min. Samples were centrifuged at 140,000 gmax for 30 min at 4°C and the solubilized material was analysed on 10% polyacrylamide gels. Gel preparation, electrophoresis and staining were as previously described (Gray *et al.*, 1978) except that gels were 6 cm rather than 10 cm in length and electrophoresis was at a constant current of 2 mA/gel for about 1 h 40 min. Gels containing 50 and 250 μg respectively of protein were stained with Coomassie brilliant blue for protein and by the periodic acid Schiffs (PAS) procedure for carbohydrate. Gels were calibrated for mol.wt using ovalbumin (40,000), bovine serum albumin (68,000), β-galactosidase (130,000) and myosin (200,000). Skeletal muscle myosin was isolated from guinea-pig back muscle by the method of Weeds & Hartley (1968).

RESULTS

Differential centrifugation

The purpose of differential centrifugation was to isolate from the homogenate a crude plasma membrane fraction which could later be purified by density gradient centrifugation. The scheme eventually adopted was a compromise between yield and purity. The distribution of the various marker

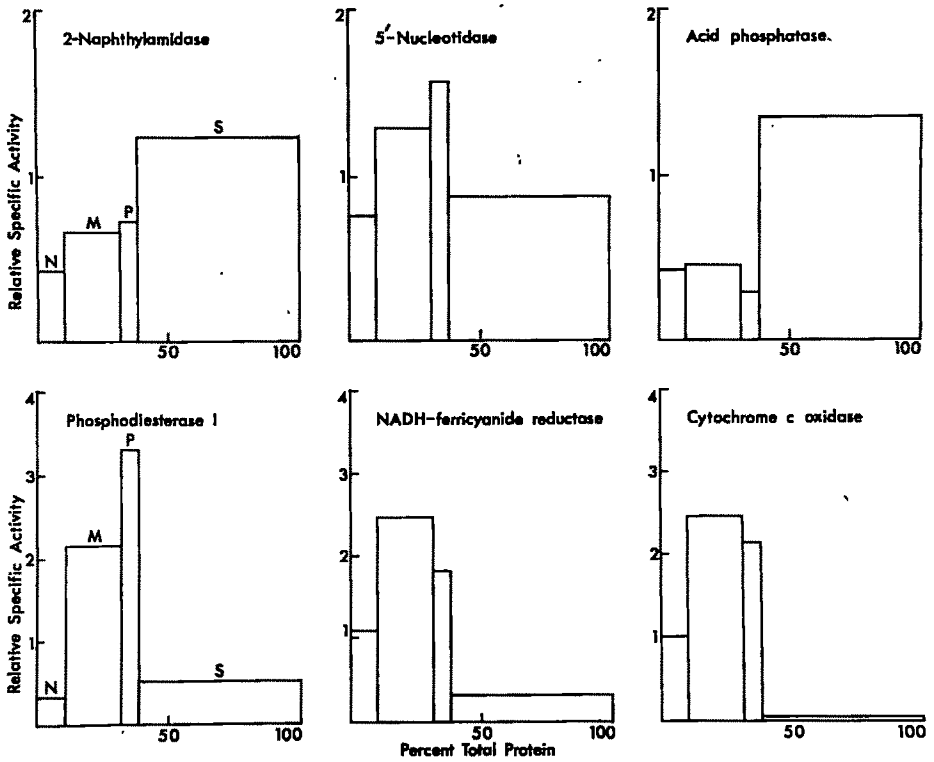

FIGURE 1. Differential fractionation of epidermal homogenate. Fraction N (700 gmax pellet); Fraction M (35 000 gmax pellet); Fraction P (160 000 gmax pellet); fraction S (soluble).

enzymes used in this work is plotted in Fig. 1 according to the classical method of de Duve (1971). It is presented in terms of the 700 gmax (fraction N), 35,000 gmax (fraction M), 160,000 gmax (fraction P) and soluble fraction (fraction S). In this representation, the specific activity of all enzymes in the homogenate is set at 1; the height of the blocks give the relative purification achieved over the homogenate (relative specific activity) and the surface area of the blocks (relative specific activity × % protein) is the percentage of the enzyme recovered in the fraction.

A feature of epidermis is that a large fraction of most marker enzymes exist in a soluble form. For acid phosphatase, 82% of the total activity is soluble. For the other enzymes tested the figures are: 2-naphthylamidase (77%), 5'-nucleotidase (55%), phosphodiesterase I (28%), NADH-ferricyanide reductase (25%) and cytochrome c oxidase (3%).

Density gradient separation

Separation of fraction M on a sucrose density gradient produced peaks of protein at sucrose molarities of 0·7 mol/l (fraction I), 1·25 mol/l (fraction II) and 1·9 mol/l (fraction III) (Fig. 2). Fraction I was enriched in plasma membrane marker enzymes and also contained a small amount of NADH-ferricyanide reductase: this fraction contained almost no cytochrome c oxidase. Fraction II was enriched in NADH-ferricyanide reductase and cytochrome c oxidase, while fraction III contained a large amount of protein with relatively small amounts of enzyme. The relative specific activities of the various enzymes are listed in Table 1.

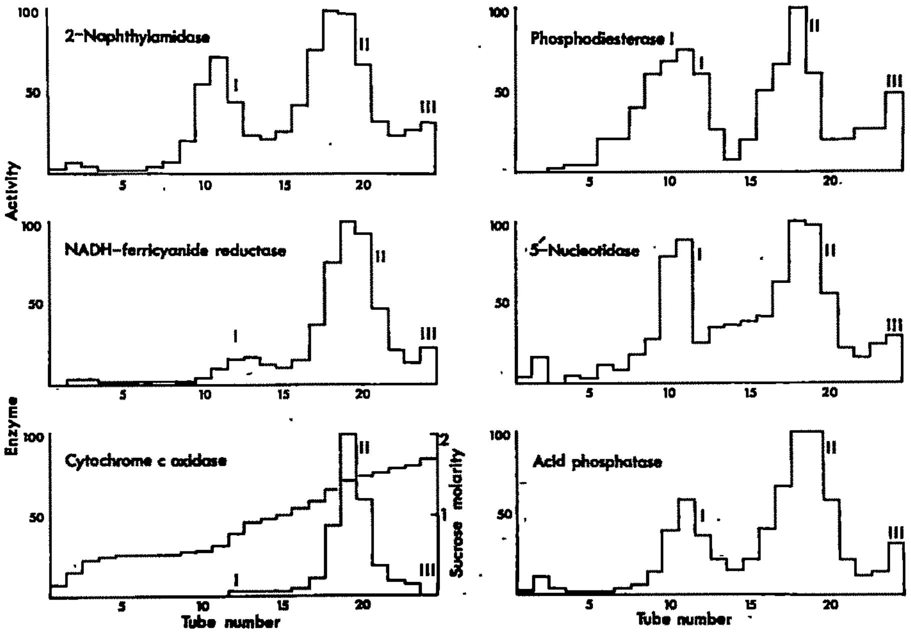

FIGURE 2. Fractionation of Malpighian cell membranes by isopycnic zonal centrifugation on a step gradient of sucrose in 5 mM tris-HCl pH 7·4. Maximum values of enzymes are arbitrarily assigned a value of 100 in each case.

TABLE 1. Relative specific activities of marker enzymes in fractions obtained by zonal centrifugation

	Fraction No.		
	I	II	III
Sucrose density	1·09	1·16	1·3
Sucrose molarity	0·7	1·25	1·9
2-Naphthylamidase	7·8 (15)	1·6 (2)	0·7 (1)
Phosphodiesterase I	10·1 (10)		0·3 (1)
5'-Nucleotidase	6·7 (3)		0·2 (1)
Acid phosphatase	4·6 (4)		0·3 (1)
NADH-ferricyanide reductase	2·7 (17)	5·3 (3)	0·6 (2)
Cytochrome c oxidase	0·7 (7)		0·4 (1)

Number of observations in parentheses.

Electron microscopy

An electron micrograph of fraction I is shown in Fig. 3. This fraction consists almost entirely of smooth-surfaced vesicular particles, 0·1–0·4 μm in diameter. Fraction II contained mitochondria, rough endoplasmic reticulum as well as smooth-surfaced vesicles and debris. Fraction III consisted chiefly of tonofilaments, nuclear material, desmosomes and a small amount of membrane material.

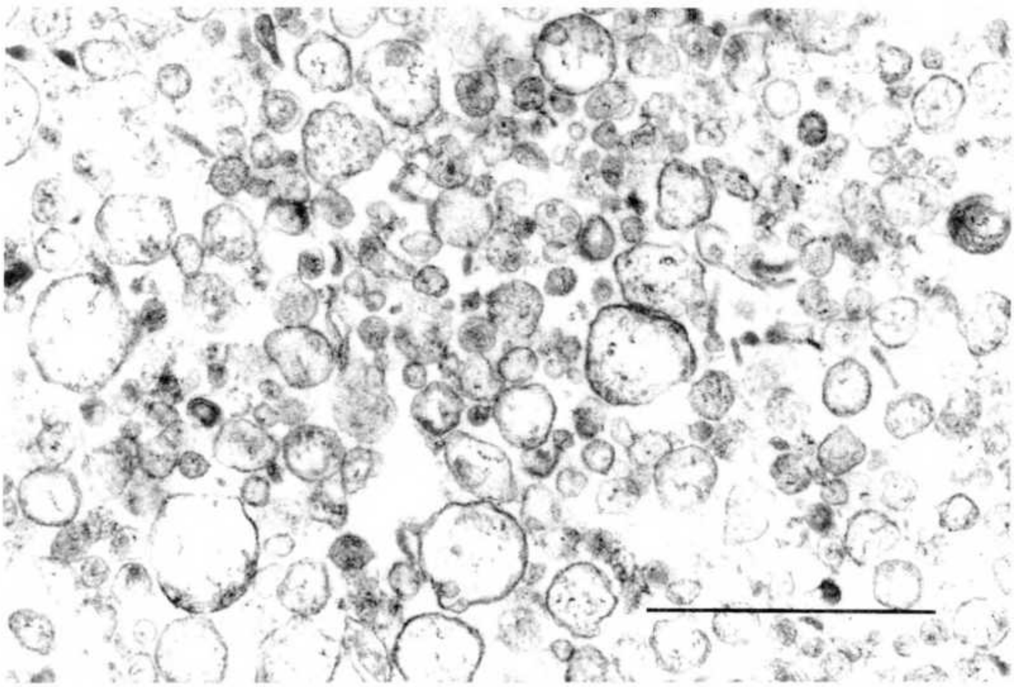

FIGURE 3. Electron micrograph of a plasma membrane fraction (Fraction I) from pig epidermis (×40,000). Scale line represents 1 μm.

Protein composition

The plasma membrane fraction (fraction I) was completely solubilized by 2% SDS, 10 mM mercaptoethanol at 100°C. Figure 4 compares the protein composition of the plasma membrane fraction with that of other subcellular fractions after electrophoresis in 10% polyacrylamide gels. At least 11 polypeptide bands were detected in the plasma membrane fraction (Fig. 4a). Band 1 (mol.wt 137,000) was diffuse and was present in variable amounts. Band 2 (mol.wt 120,000–90,000) contained at least four closely migrating polypeptides and was enriched compared with the other particulate fractions (Fig. 4b and c). When gels were stained for carbohydrate by the PAS procedure, a diffuse pink band corresponding to the band 2 proteins was observed. This band, therefore, contained glycoproteins. In some preparations a faint diffuse PAS reaction was also observed in the region of band 1. Because glycoproteins bind relatively less SDS than non-glycosylated proteins, they migrate anomalously during electrophoresis. The molecular weights of these polypeptides should therefore be regarded as only approximations. Band three (mol.wt 79,000) was also enriched in the plasma membrane fraction. Bands 4, 5, 6 and 7 (mol.wt 63,000, 57,000, 52,000 and 46,000, respectively) were present in about equal amounts but were always minor components.

Polypeptides in this molecular weight range were present at higher concentration in the other subcellular fractions. Fraction P was enriched in bands equivalent to bands 4 and 7 together with another polypeptide (mol.wt 72,000). These three polypeptides and a band of mol.wt 58,000 were the major components of the N fraction (Fig. 4c).

The major protein component of the soluble fraction (Fig. 4d) had an apparent molecular weight of 64,000, was diffuse but always appeared to migrate as a single component. It was not clear whether this corresponded to any of the bands of similar molecular weight detected in the plasma membrane

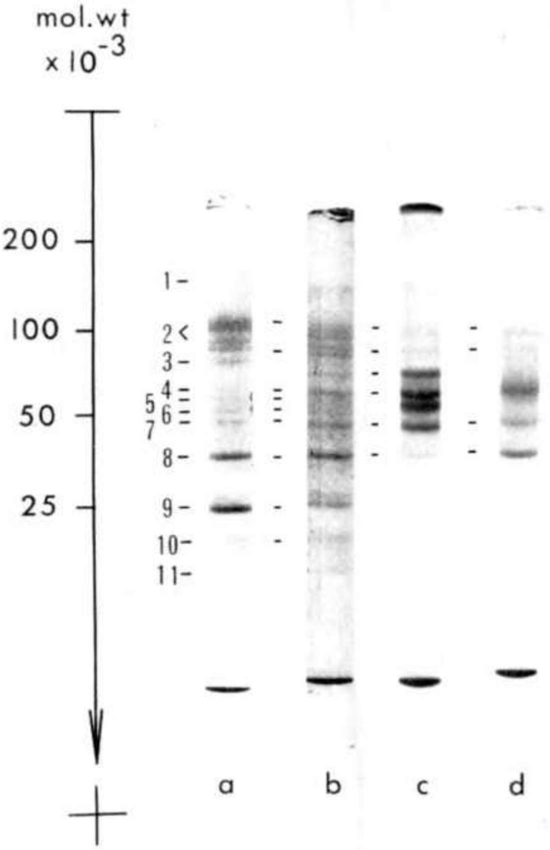

FIGURE 4. Protein composition of plasma membrane fraction (Fraction I) and other subcellular fractions from pig epidermis. Samples solubilised with 2% SDS, 10 mM 2-mercaptoethanol were analysed on 10% polyacrylamide gels. About 50 μg of protein was applied to each gel. (a) fraction I (plasma membrane fraction). (b) Fraction P (160, 000 gmax pellet). (c) Fraction N (700 gmax pellet). (d) Fraction S (soluble fraction).

and particulate fractions. However, two polypeptides in this soluble fraction had a similar electrophoretic mobility to bands 7 and 8 in the plasma membrane fraction. Bands 8 and 9 (mol.wt 39,000 and 24,000) were major components of the plasma membranes. Polypeptides of lower molecular weight were variable but were always minor components and included bands 10 and 11 (mol.wt 19,000 and 15,000 respectively).

Lipid composition

Several preparations of plasma membranes were combined to obtain sufficient lipid material (1–2 mg) for analysis. The lipid composition is shown in Table 2 which also includes, for comparative purposes, the lipid composition of a preparation of pig granular cell membranes (Gray *et al.*, 1978) and of a preparation of pig Malpighian cells (Gray & Yardley, 1975a). The plasma membrane preparation had a

TABLE 2. Lipid compositions of plasma membrane fractions isolated from pig cells and whole Malpighian cells

	Plasma membrane fraction from Malpighian cells	Plasma membrane fraction from granular cells	Malpighian cells
Phosphatidylethanolamine	13·7	4·4	11·9
Diphosphatidylglycerol	1·9	ND	2·1
Phosphatidyl-(N-acyl)-ethanolamine	1·8	5·5	0·8
Lyso-phosphatidyl-(N-acyl)-ethanolamine	ND	0·7	tr
Phosphatidylserine	6·3	0·8	6·5
Phosphatidylinositol	5·1	0·4	5·6
Phosphatidic acid	ND	0·5	0·8
Phosphatidylcholine	21·5	9·3	23·6
Sphingomyelin	18·1	9·2	10·7
Unidentified phospholipid	8·8	—	—
Glucosylceramides	0·5	9·6	7·3
Cholesterol	10·4	26·7	9·0
Cholesterol esters	0·2	0·4	0·9
Ceramides	2·2	11·4	0·9
Fatty acids	3·8	16·0	6·7
Triacylglycerols	5·4	2·3	7·5
Hydrocarbons	1·5	1·8	2·4
Unidentified neutral lipid	—	—	3·3
Cholesterol : phospholipid (molar ratio)	0·26	1·68	0·28

Values are expressed as a percentage, by weight, of total lipids. ND, not determined, insufficient material. tr, trace.

protein to lipid content of 3:2 by weight. The major lipids were phospholipids (76% of total lipid) and most of the neutral lipids were accounted for by cholesterol, triacylglycerols, fatty acids and some ceramides. The preparation contained very little glycosphingolipid (glucosylceramide).

The amounts of glycosphingolipid in the filtered epidermal homogenate and in the 700 *g* pellet were also estimated. The glycosphingolipids accounted for 9% of the total lipids in the filtered homogenate and 13% of the total lipids in the 700 *g* pellet. Examination of the 700 *g* pellet by electron microscopy showed that it was rich in desmosomes.

DISCUSSION

Though the starting material for the preparation was whole epidermis, we believe that the plasma membrane fraction was derived only from Malpighian cells for the following reasons. Firstly, the fraction was collected in sucrose having a density of 1·09 following isopycnic centrifugation, whereas membrane prepared by us (Gray *et al.*, 1978) from granular cells had a buoyant density of 1·195 corresponding to a sucrose molarity of 1·50. Thus, any membrane removed from granular cells during the homogenization of epidermis would have appeared in fraction II (see Fig. 2). Secondly, the membrane prepared from granular cells in the same medium existed as large sheets.

Our membrane preparations were enriched in the putative plasma membrane markers 2-naphthylamidase, phosphodiesterase I and 5′-nucleotidase. Acid phosphatase was also enriched, and this enzyme has been reported to be associated with plasma membrane (Robinson, 1969). The preparations were not contaminated with mitochondrial material as judged by the almost complete absence of the mitochondrial marker, cytochrome c oxidase. It is more difficult to be precise about any contamination with endoplasmic reticulum, because no good marker for this membrane system has yet been found in epidermis. Glucose-6-phosphatase and aryl sulphatase C are commonly used as markers for endoplasmic reticulum in other tissues, but we have been unable to detect these enzymes in pig epidermis (Gray & Yardley, 1975b). The enzyme which catalyses the transfer of an electron from NADH to the ferricyanide ion has been used as an endoplasmic reticulum marker by Thom *et al.* (1977) and by Ferber *et al.* (1972). The enzyme is stable, extremely active in epidermis and easily measured and thus fulfils three of the criteria for an enzyme marker. It does, however, suffer from the disadvantage shared with most enzymes found in epidermis, of being partially soluble. Furthermore, the enzyme has been shown to attach itself to both natural and artificial membranes (Leroux, Torlinski & Kaplan, 1977) and recently, NADH-ferricyanide reductase has been detected in a highly purified preparation of mouse liver plasma membrane (Goldenberg, Crane & Morré, 1979). Thus the presence of NADH-ferricyanide reductase in the plasma membrane preparation may be due to intrinsic enzyme, or it could reflect the presence of adsorbed soluble enzyme or contamination by endoplasmic reticulum.

The isolated plasma membranes had a complex protein composition containing polypeptides whose molecular weight ranged between 137,000 and 13,000. Their protein composition was also quite distinct from that reported for other plasma membrane fractions isolated from the epidermis. We have previously described the protein composition of plasma membranes isolated from trypsin-treated granular cells (Gray *et al.*, 1978). Granular cell membranes analysed in the presence of 8 M urea, 5% SDS contained major polypeptides in the mol.wt range 56,000 to 50,000. However, polypeptides in this molecular weight range were only minor components of the plasma membranes described here. Conversely, the major glycoproteins (mol.wt 120,000 to 90,000) were only minor components of the isolated granular cell membrane. Two other major polypeptides of the present plasma membrane preparation (mol.wt 39,000 and 24,000) were not previously detected in the granular cell membranes. These various protein components may become degraded during the extensive plasma membrane modifications that accompany keratinization. Alternatively, their absence from granular cell membranes may be due to the trypsinization used to isolate granular cells.

The protein composition of the plasma membranes described here can also be compared with that of desmosomes isolated from cow snout epidermis (Skerrow & Matoltsy, 1974; Drochmans *et al.*, 1978). The large (mol.wt 230,000 and 210,000), filamentous, 'spectrin-like' proteins which were major components (28% of total protein) of acid-treated desmosomes (Skerrow & Matoltsy, 1974) were not detected in the present plasma membrane preparations. They may be unique to desmosomal areas of the plasma membrane. The major proteins in the desmosome-tonofilament complexes isolated by Drochmans *et al.* (1978) had apparent mol.wt of 68,000, 60,000, 58,000, 55,000 and 48,000: the four smaller polypeptides may be equivalent to bands 4, 5, 6 and 7 present as minor components of our plasma membrane preparations. The 68,000 mol.wt polypeptide which may be a constituent only of those tonofilaments associated with desmosomes was not present in our plasma membranes. However, a polypeptide of similar mol.wt (72,000) was detected in the crude particulate fractions. Actin (subunit mol.wt 43,000) is associated with plasma membranes isolated from many cell types (Tilney & Mooseker, 1971) but was not present in isolated desmosomes. However, the mol.wt of the band 8 polypeptide (39,000) in our plasma membranes is close to that of actin. Membrane bound actin may therefore be unique to non-desmosomal areas of the plasma membrane.

Comparison of the glycoprotein composition of our plasma membrane preparations with earlier analyses of desmosomal glycoproteins is complicated for two reasons. First, glycoproteins migrate anomalously during electrophoresis in SDS (Bretscher, 1971) so that molecular weights can only be approximated and second, different electrophoretic systems were used. However, the complex glycoprotein band (apparent mol.wt 120,000–90,000) of the present plasma membranes may correspond to the glycoprotein band (mean apparent mol.wt 120,000) detected in acid-treated desmosomes. A strongly stained glycoprotein (apparent mol.wt 137,000) was present mainly in our crude particulate fractions but was only weakly stained if at all in the purified plasma membranes. A glycoprotein of similar apparent molecular weight was a major glycoprotein of isolated desmosomes (Skerrow & Matoltsy, 1974; Drochmans *et al.*, 1978). This glycoprotein may therefore be preferentially located in junctional areas of the plasma membrane. Clarification of this point will require the isolation of desmosomal and non-desmosomal plasma membrane fractions from the same tissue source and their comparison in the same electrophoretic system.

Characteristically the lipids in the plasma membrane preparation were relatively rich in sphingomyelin compared with the lipids from other epidermal cell organelles (Gray & Yardley, 1975a) and with the lipids of the whole cells. However, we were surprised to find so little glucosylceramide in the preparation especially as in general the glycosphingolipids are located predominantly in the plasma membranes of mammalian cells (Hakomori, 1975) and in pig epidermis glucosylceramides account for about 7–9% of the total lipid in the cells. It seems likely that much of the glucosylceramide is associated with the desmosome areas of the membrane and these were not present in our membrane preparations but were plentiful in the material in the 700 *g* pellet. As the proteins which Drochmans *et al.* (1978) had identified as components of epidermal desmosomes were not found in the membrane preparation either, we conclude that the preparation was probably derived from the non-desmosomal areas of the plasma membranes of Malpighian cells.

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Studies on human epidermal Langerhans cells

III. INDUCTION OF T LYMPHOCYTE RESPONSE TO NICKEL SULPHATE IN SENSITIZED INDIVIDUALS

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SUMMARY

Human epidermis from nickel sensitive patients was separated from dermis by means of a suction blister device and dissociated with trypsin. The epidermal cell suspensions obtained contained 3–5% Langerhans cells as judged by immunofluorescence staining of the cells with a rabbit anti-DR antiserum. The epidermal cells were co-cultured with purified autologous T lymphocytes with or without nickel sulphate. A strong proliferative T lymphocyte response to nickel sulphate was obtained provided epidermal cells were also present. Pretreatment of the epidermal cells with anti-DR antiserum abolished or greatly reduced the response.

These data indicate that epidermal cells are able to present nickel sulphate to T lymphocytes in an immunogenic way. Since the responsible cells were DR antigen positive, it is highly probable that the cells responsible for these functions are the Langerhans cells.

The epidermal Langerhans cells constitute only a minor cell population of the human epidermal cells, but nevertheless seem to play an important role in the immune system of the skin.

They have Fc and C3 receptors (Stingl *et al.*, 1977) and express DR antigens (Klareskog *et al.*, 1977; Rowden, Lewis & Sullivan, 1977; Stingl *et al.*, 1978a), similar to peripheral blood macrophages. The D/DR antigens have been shown to be mainly responsible for the proliferative response of T cells when confronted with allogeneic cells *in vitro*, i.e. the mixed lymphocyte culture (MLC) interaction (Thorsby *et al.*, 1978). Recently we demonstrated the ability of HLA-DR positive Langerhans cells to stimulate allogeneic T lymphocytes (Braathen & Thorsby, 1980). Macrophage D/DR apparently participate in the activation of T cells with foreign antigens such as PPD (Bergholtz & Thorsby, 1977; 1978; Sönderstrup Hansen *et al.*, 1978), herpes simplex virus (Berle & Thorsby 1980a) and haptens such as TNP (Thorsby & Nousiainen, 1979).

Studies on guinea-pigs have shown that the Ia positive Langerhans cells in this species can substitute for macrophages in antigen presentation to T cells (Stingl *et al.*, 1978b). In humans we were recently able to demonstrate that epidermal Langerhans cells can substitute for macrophages in

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inducing PPD specific, as well as herpes simplex virus antigen and live virus specific, responses of T cells from sensitized donors (Braathen & Thorsby, 1980; Braathen *et al.*, 1980).

The Langerhans cells can selectively take up various contact allergens, among them also nickel (Shelley & Juhlin, 1977). A close apposition to mononuclear cells was frequently seen at sites of positive allergic contact dermatitis but not in contact primary irritant reactions (Silberberg, 1971, 1972, 1973). Both these findings indicate a role of the Langerhans cells in contact allergic dermatitis.

Previous studies have shown that mononuclear cells separated from sensitized individuals respond to nickel (Svejgaard *et al.*, 1978; Forman & Alexander, 1972; Hutchinson, Raffle & MacLeod, 1972; Gimenez-Camaraza *et al.*, 1975; MacLeod, Hutchinson & Raffle, 1970; Millikan, Conway & Foote, 1973). Here we report that purified T cells from sensitized donors will not alone respond to nickel but will do so when nickel is present together with autologous human epidermal cells expressing the D/DR antigens. The responsible cells are in all probability the Langerhans cells.

MATERIALS AND METHODS

Cell donors

Cell donors were unrelated inpatients of the Department of Dermatology and healthy staff members. The patients had contact allergic dermatitis with a positive epicutaneous patch test to nickel. The test was performed according to the European Standard series with 5% nickel sulphate in petrolatum, and read after 48,72 and 96 h. All the patients reacted with erythematous infiltration and papules or vesicles.

Epidermal cells (Ec)

Epidermis of volunteers was separated from dermis at the basement membrane *in vivo* by means of a suction blister device (Kiistala, 1968). The sheets of epidermis were then dissociated by means of trypsin essentially as described by Stingl *et al.* (1977). After washings, the cells were resuspended in Medium RPMI 1640 with L-glutamine (Gibco Bio-Cult, Glasgow, Scotland) supplemented with penicillin, streptomycin and 20% normal human serum. Viability was checked by trypan blue exclusion and ranged from 80 to 95%, but was usually more than 90%. The cell suspensions contained 3–5% Langerhans cells, as judged by immunofluorescence staining of the cell suspensions with a rabbit anti-DR antiserum. The staining was performed by first incubating the cell suspensions with the anti-DR antiserum at 4°C for 30 min. and then, after two washings, the cells were incubated for another 30 min at 4°C with an FITC-conjugated swine anti-rabbit antiserum (DAKO-immunglobulin, Copenhagen, Denmark). After final washings the cells were counted in an immunofluorescence microscope.

Rabbit anti-DR antiserum and normal rabbit serum (NRS)

The anti-DR antiserum preparation is described elsewhere (Solheim *et al.*, 1978). It was complement inactivated (56°C for 30 min) and absorbed with pooled human erythrocytes before use.

Nickel sulphate solution

Nickel (II) sulphate ($NiSO_4 \times 6H_2O$) 'zur Analyse' with molecular weight 262·86 was obtained from Merck AG, Darmstadt, W. Germany, Art. no. 6727. A stock solution of 1% nickel sulphate in 0·9% saline was prepared and additional dilutions were made with Medium RPMI 1640. Usually three dilutions were used in the experiments; 1:25, 1:50 and 1:100, but in some experiments higher dilutions were also used.

Peripheral blood mononuclear (PBM) cells

Peripheral blood mononuclear cells were separated by the Ficoll-Isopaque method (Bøyum, 1976) from defibrinated blood by means of Lymphoprep (Nyegaard & Co., Oslo, Norway).

T Lymphocytes

Purified T lymphocytes were prepared from isolated mononuclear cells suspended in Medium RPMI 1640 supplemented with penicillin, streptomycin and 20% normal human serum. The cell suspensions were incubated in 25 cm^2 tissue culture flasks (Falcon no. 3013) at 37°C in a humid 5% CO_2 atmosphere. The non-adherent cells were then removed by washings with culture medium and mixed with an equal volume of a 1% suspension of aminoethylisothiouronium bromide (AET) treated sheep erythrocytes.

The mixture was centrifuged at 200*g* for 5 min, the pellet gently resuspended and the cell suspension layered on Lymphoprep and centrifuged for 20 min at 800*g*. The sheep erythrocytes in the pellet were haemolysed by incubation with fresh autologous serum for 15 min at 37°C (Albrechtsen, Solheim & Thorsby, 1977). After washing and resuspension in Medium RPMI 1640, this procedure was performed once more. The remaining T cell enriched cells (double-rosetted) were washed twice before being used. They contained less than 1% latex ingesting cells, less than 5% Ig-positive cells and more than 75% E-rosette forming cells.

Preparation of adherent cells/macrophages (Mø)

The remaining adherent cells in the tissue culture flasks were incubated with medium RPMI 1640 supplemented with 30% normal human serum and 3·33 mg/ml EDTA for 90 min on ice before being removed by forcible pipetting. After two washings at 4°C the cells were kept on ice until used. The viability of these cells was usually more than 95% as determined by trypan blue exclusion. More than 80% ingested latex particles.

Cell cultures

Cell cultures were incubated in round-bottomed microtiter plates (Model 15-MRC-96, Linbro Scientific, Inc., Hamden, Conn., U.S.A.) in a humid 5% CO_2 atmosphere at 37°C for 5 days. 5×10^4 T lymphocytes were co-cultured with various numbers of epidermal cells or adherent cells with and without 20 μl nickel sulphate in various dilutions. Each well contained 170 μl Medium RPMI 1640 supplemented with antibiotics and 20% normal human serum. ^{3}H-Thymidine was added 18 h before harvesting, which was performed with a semi-automatic multiple cell culture harvester (Skatron, Lierbyen, Norway). Incorporation was measured by means of a liquid scintillation counter and expressed as mean cpm ± SE of triplicates.

Peroxidase staining

Peroxidase staining of epidermal cell suspensions was performed with cytocentrifuge (Shandon Scientific Co., Ltd, London) preparations as described by Kaplow (1965).

Treatment of epidermal cells with anti-DR antisera or normal rabbit serum

Twenty microlitres anti-DR antiserum or normal rabbit serum (previously absorbed with pooled human erythrocytes) was added to the wells containing epidermal cells and allowed to react for 1 h at room temperature before T cells and nickel sulphate were added.

RESULTS

Staining of epidermal cell suspensions with peroxidase

The results to be reported depend heavily on the eventual monocyte-macrophage contamination of the epidermal cell suspensions used. For this reason, cytocentrifuge preparations of three epidermal cell suspensions were stained with peroxidase to detect monocytes. More than 1000 cells from each suspension were examined, but no stained cells were detected in any of them.

Epidermal cell-dependent autologous T lymphocyte response to nickel sulphate

In these experiments epidermal cells (Ec) were co-cultured with autologous T lymphocytes and various dilutions of nickel sulphate. The results of five typical experiments are recorded in Table 1. A strong Ec-dependent T cell response to nickel sulphate was obtained as compared to the controls. T cells cultured with adherent cells (Mø) and nickel sulphate showed little response, while T cells cultured without Ec showed no response to nickel sulphate. Ec cultured with nickel sulphate alone and Ec cultured with T cells without nickel sulphate did not show any stimulation. T cells from healthy staff members cultured with nickel sulphate and autologous adherent cells did not show stimulation. These data demonstrate that epidermal cells can induce a T cell response to nickel sulphate in sensitized donors.

TABLE 1. Epidermal cell-dependent T lymphocyte response to $NiSO_4$ *in vitro*

	T cells*		T+Ec†	
$NiSO_4$	−‡	+‡	−	+
Exp. no.				
1	221± 7§	453± 59	171± 39	12299±2592
2	406± 26	526±179	180± 22	10788± 700
3	346±156	159± 89	124± 38	1202± 159
4	160± 58	84± 25	194± 78	2601± 652
5	274± 88	186± 28	109± 3	3266± 442

* 5×10^4 T cells.

† 5×10^4 epidermal cells.

§ Mean cpm ± SE.

‡ Absence or presence of $NiSO_4$ dilution 1:10 000.

Influence of the concentration of nickel sulphate on the epidermal cell-dependent T cell response

In each experiment, several dilutions of nickel sulphate were added to the wells, ranging from 1:2500 to 1:400,000. Usually, however, 1:2500, 1:5000 and 1:10,000 dilutions were used. The results of two typical experiments are shown in Table 2. The response was generally higher with higher concentrations of nickel sulphate. The dilution 1:25000 might have been slightly toxic, while the dilutions 1:5000 and 1:10000 seemed to give optimal responses.

Influence of the number of epidermal cells added to the cultures on the T cell response to nickel sulphate

In these experiments increasing numbers of Ec were added to 5×10^4 autologous T cells and nickel sulphate. The results of two typical experiments are recorded in Fig. 1. A significant response was obtained with as few as $2{\cdot}5 \times 10^3$ epidermal cells. Increasing numbers of Ec added increased the nickel sulphate response and maximum response was obtained with the highest tested number of 100×10^3 epidermal cells added.

TABLE 2. Influence of the nickel sulphate concentrations on the epidermal cell dependent T cell response

Exp. no.	NiSO$_4$ dilutions*	T cells†	T+Ec		
			2§	5	10
	1×10^{-4}	453 ± 59‡		12299 ± 2592	13681 ± 972
	2×10^{-5}	134 ± 20		5710 ± 673	7074 ± 648
1	1×10^{-5}	127 ± 82		4158 ± 604	4491 ± 780
	5×10^{-6}	533 ± 187		2055 ± 387	3140 ± 218
	$2{\cdot}5 \times 10^{-6}$	544 ± 185		828 ± 462	1862 ± 966
	4×10^{-4}	480 ± 56	2593 ± 470	12031 ± 1431	15892 ± 3442
2	2×10^{-4}	163 ± 16	18112 ± 243	30277 ± 1438	37649 ± 2314
	1×10^{-4}	526 ± 179	13581 ± 2904	27003 ± 2278	31739 ± 2039

† 5×10^4 T cells.
* Nickel sulphate dilutions added to the wells.
§ Number of epidermal cells $\times 10^{-4}$.
‡ Median cpm ± SE of triplicates.

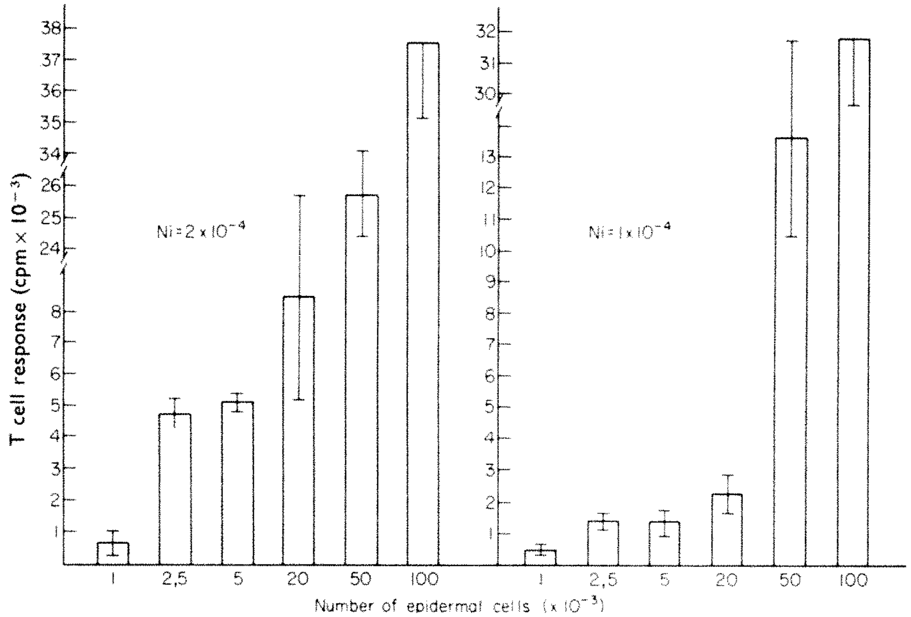

FIGURE 1. Influence of the number of epidermal cells (Ec) added to the cultures on the T cell response to nickel sulphate dilutions 2×10^{-4} and 1×10^{-4}. Values are in mean cpm ± SE.

Kinetics study of the epidermal cell-dependent T cell response to nickel sulphate

In these experiments cultures were harvested every 2 days from the third until the eleventh day. The results of two typical experiments are given in Fig. 2. No significant response was obtained on day 3 and day 11. A peak response was obtained on day 7 in all the experiments.

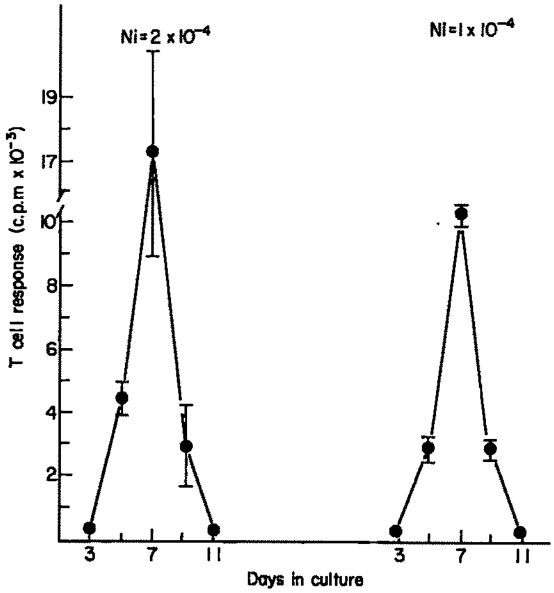

FIGURE 2. Kinetics study of the epidermal cell dependent autologous T lymphocyte response to two nickel sulphate dilutions, expressed as mean cpm ± SE.

TABLE 3. Effect of treatment of epidermal cells with anti-DR antiserum or normal rabbit serum on the autologous T lymphocyte response to $NiSO_4$

			T cells plus $NiSO_4$ and:		
Exp. no.	No. of Ec	$NiSO_4$ dilutions	Untreated Ec	NRS-treated* Ec	Anti-DR† treated Ec
	2×10^4	2×10^{-4}	18112 ± 243‡	13877 ± 1497	2219 ± 465
	5×10^4	2×10^{-4}	30277 ± 1438	32660 ± 1075	4711 ± 909
	10×10^4	2×10^{-4}	37649 ± 2314	31871 ± 1149	5192 ± 1358
1					
	2×10^4	1×10^{-4}	13581 ± 2904	9398 ± 1780	1337 ± 513
	5×10^4	1×10^{-4}	27003 ± 2278	21959 ± 863	2702 ± 362
	10×10^4	1×10^{-4}	31739 ± 2038	26960 ± 3288	2440 ± 617
2	2.5×10^4	2×10^{-4}	8441 ± 171	6394 ± 512	393 ± 115
	2×10^4	2×10^{-4}	2770 ± 564	2207 ± 337	385 ± 175
3	5×10^4	2×10^{-4}	5082 ± 299	7704 ± 181	372 ± 53
	10×10^4	2×10^{-4}	5701 ± 119	8669 ± 923	1780 ± 388

* Epidermal cells treated with normal rabbit serum.
† Epidermal cells treated with anti-DR antiserum.
‡ cpm ± SE of triplicates.

Effect of treatment of epidermal cells with rabbit anti-DR antiserum or normal rabbit serum on the T cell response to nickel sulphate

Anti-DR antiserum or normal rabbit serum (NRS) was added to wells containing epidermal cells and allowed to react for 1 h at room temperature before T cells and nickel sulphate were added. The results of three typical experiments are presented in Table 3. The response obtained in wells with NRS added was similar to the response obtained with untreated Ec. In contrast, the response in wells with anti-DR antiserum was abolished or greatly reduced, indicating that the nickel sulphate presenting cells are DR antigen positive.

DISCUSSION

The present study demonstrates that human epidermal cells are able to induce a T lymphocyte response to nickel sulphate in sensitized individuals. We also present evidence that the cells responsible for this function carry the HLA-DR molecules.

In previous studies we showed that the Langerhans cells most probably are responsible for the allo-activating capacity of human epidermal cells (Braathen & Thorsby, 1980). Furthermore, we presented evidence that they can present the bacterial antigen purified protein derivative (PPD) of tuberculin (Braathen & Thorsby, 1980) and herpes simplex virus (Braathen *et al.*, 1980) in an immunogenic way to T lymphocytes. These observations are in accordance with similar data obtained in studies on guinea-pigs (Stingl *et al.*, 1978b).

Three major types of cells are present in the epidermal cell suspensions we used; keratinocytes, melanocytes and Langerhans cells. The presence of other cells from dermis contaminating the cell suspensions is unlikely, since the epidermal sheets we used are separated from dermis at the dermo-epidermal junction (Kiistala, 1968). No monocytes were present, as indicated by the absence of peroxidase positive cells in the suspensions.

No significant stimulation was obtained in cultures containing only T cells and nickel sulphate. Little stimulation was obtained in cultures containing T cells, autologous adherent cells (Mø) and nickel sulphate as compared to the cultures containing T cells, epidermal cells and nickel sulphate. Further studies are being performed to elucidate this. The response increased with increasing numbers of epidermal cells added, and maximum stimulation was obtained with 1×10^5 epidermal cells present in the wells, i.e. a T/Ec ratio of 1:2. The kinetics study showed peak stimulation on day 7, which is in accordance with the results obtained by Svejgaard *et al.* (1978), using peripheral blood mononuclear cells.

Nickel is a frequent cause of allergic contact dermatitis. The diagnosis is made on the basis of a positive patch test, but in an acute phase of the dermatitis, testing may exacerbate the dermatitis or give non-specific false positive reactions (Fisher, 1967), and therefore be undesirable. An *in vitro* test is, in such cases, an advantage. All the patients showed a strong reaction to the epicutaneous patch test with nickel sulphate, but the *in vitro* epidermal cell-dependent nickel response varied considerably.

Previous studies have shown that lymphocytes from patients with a positive nickel patch test and a contact dermatitis caused by nickel respond to nickel sulphate *in vitro* (Forman & Alexander, 1972; Gimenez-Camarasa *et al.*, 1975; Hutchinson, Raffle & MacLeod, 1972; MacLeod, Hutchinson & Raffle, 1970; Millikan *et al.*, 1973; Svejgaard *et al.*, 1978). Gimenez-Camarasa *et al.* (1975) demonstrated two groups of nickel-sensitive patients, one group responding to low and one mainly to high nickel sulphate concentrations.

Previous reports have claimed that nickel may cause non-specific lymphocyte transformation (Aspegren & Rorsman, 1962; Pappas, Orfanos & Bertram, 1970). Other results also seem to support such a notion (Svejgaard *et al.*, 1978); nickel sulphate could be a weak non-specific mitogen. In the patients with nickel contact dermatitis, however, the nickel clearly acts as an antigen causing damage to the skin. Nickel is generally believed to be a hapten which bound to a carrier molecule becomes immunogenic. If true, the carrier could be components of serum or the cells' surfaces. Autoradiograph studies have demonstrated direct binding of nickel salts to the lymphocyte cell surface (Hutchinson, MacLeod & Raffle, 1975). Previous studies from this laboratory have shown that the T cell responses to PPD and herpes simplex virus are macrophage-dependent and restricted by the self HLA-D/DR molecules of the T cell donor (Bergholtz & Thorsby, 1977; Bergholtz & Thorsby, 1978; Berle & Thorsby, 1980b). Studies are in progress to investigate whether this is also true for the T cell responses where Langerhans cells can substitute for macrophages.

No or only minimal responses were seen in cultures when anti-DR antiserum was added, while addition of normal rabbit serum (NRS) did not significantly reduce the responses. This indicates that the nickel sulphate presenting function was performed by HLA-DR positive cells. In epidermis, only Langerhans cells (Klareskog *et al.*, 1977; Rowden, Lewis & Sullivan, 1977; Stingl *et al.*, 1978a) and the majority of the few so-called indeterminate cells have HLA-DR antigens (Rowden, Phillips & Lewis, 1979). A relationship exists between these indeterminate cells and the Langerhans cells and it was recently proposed that when Langerhans cells emigrate in response to haptenic stimulation, the indeterminate cells immigrate to restore the status quo (Rowden *et al.*, 1979). Indeterminate cells, therefore, may well be immature Langerhans cells.

It is therefore highly probable that the nickel presentation is performed by the Langerhans cells in the epidermal cell suspensions.

It has been proposed that Langerhans cells might capture antigenic material and thus play a role in the primary immune response (Hashimoto, 1971; Prunieras, 1969; Vernon *et al.*, 1973). The ability of Langerhans cells selectively to take up contact allergens, among them nickel, was demonstrated by Shelley & Juhlin (1977). The same authors also proposed that the Langerhans cells form a reticulo-epithelial trap for external contact allergens (Shelley & Juhlin 1976). The frequent occurrence of apposition of mononuclear, possibly lymphocyte, cells to Langerhans cells in contact allergic reactions (Silberberg, 1973), suggests that they cooperate, and indicates that the Langerhans cells present antigen to lymphocytes locally in the skin. Our findings support that. The Langerhans cells have also been found in dermis (Zelickson, 1965) circulating in dermal vessels, capillaries (Zelickson, 1979) and probably lymphatics (Silberberg, Baer & Rosenthal, 1976), and in lymph nodes (Gianotti Caputo & Ranzi, 1968; Jimbow, Sato & Kukita, 1969; Kondo, 1969). This indicates that the Langerhans cells possibly also transport antigenic material to dermis and lymph nodes, where they may participate in the immune response.

Previously we reported studies indicating that both PPD and herpes simplex virus may first be bound to Langerhans cells and then presented in association with self-HLA-D/DR molecules to T lymphocytes (Braathen & Thorsby, 1980, Braathen *et al.*, 1980). These events could constitute the afferent phase in the cutaneous cellular immune responses to these antigens. The results presented here may indicate a similar role of Langerhans cells in contact allergic dermatitis caused by nickel. The nickel sulphate is bound to Langerhans cells and may then be presented in association with HLA-D/DR molecules to T lymphocytes, constituting the afferent phase in the cutaneous cellular immune response to nickel. This is the object of further studies.

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Polymorphonuclear leukocyte chemotaxis in psoriasis: enhancement by self-activated serum

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SUMMARY

The chemotactic activity of polymorphonuclear leukocytes (PMNs) directed against serum was determined in a modified Boyden chamber assay. In a total of 176 experiments PMNs and sera from healthy controls were compared with PMNs and sera from patients with psoriasis. When PMNs from patients with psoriasis were confronted with psoriatic serum greatly enhanced chemotaxis was demonstrated. Non-psoriatic PMNs in the presence of psoriatic serum showed no enhancement. However, psoriatic PMNs in the presence of normal serum were chemotactically more active compared to non-psoriatic PMNs. Heat inactivation (56°C, 30 min) reduced the chemotactic activity of all sera by nearly 50%. However in psoriatic sera the enhancement of chemotaxis was still present after heat treatment.

The results indicate the presence of a functional abnormality of chemotaxis in psoriasis. This is likely to be caused by the *in situ* generation of chemotactically active fragments of complement. Experiments showing increased chemotactic activity of sera exposed to migrating PMNs support this concept.

In psoriatic epidermis polymorphonuclear leukocytes (PMNs) are constantly migrating toward the keratogenous zone. They leave their vascular environment predominantly at the tips of the papillae and rapidly gain access to the epidermal intercellular space (Pinkus & Mehregan, 1966). Although exact data are lacking, PMNs appear to be more numerous in active, eruptive psoriasis as compared to the stationary form. Indeed, the appearance of pustules during an acute relapse (e.g. psoriasis cum pustulatione after streptococcal throat infections, etc.) indicates that disease activity correlates with polymorph activation (Langner *et al.*, 1978).

PMNs are migratory cells sensitive to a variety of chemo-attractants including psoriatic scale extracts (Langhof & Muller, 1966; Tagami & Ofuji, 1976). Lazarus, Yost & Thomas (1977) found that psoriatic epidermis, unlike the normal, contains considerable amounts of serine-proteinase which activates complement and induces PMN accumulation.

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On the other hand, PMNs from patients with psoriasis were shown to be more active in migrating toward chemoattractants e.g. casein (Langner *et al.*, 1978) or endotoxin activated serum (Wahba *et al.*, 1978). In the acute or active phase of psoriasis higher chemotactic activities were found using casein as chemoattractant (Langner *et al.*, 1978). Both types of abnormality in psoriasis, the presence of chemotactic scale products and the increased chemotactic activity of PMNs may be related to the pathomechanism of the disease. So far, they have not been found in other abnormalities of the skin.

In the present study we have found that PMNs from patients with psoriasis are able to activate serum components which act as potent chemoattractants in the autologous system.

PATIENTS AND METHODS

Patients

All patients investigated were hospitalized for treatment of psoriasis. They agreed to the proposed investigations by written consent. All blood samples were taken before any therapy was started.

Preparation of PMNs

PMNs were isolated by a modification of the method described by Henson (1971). Venous blood was drawn into 1/6 volume of acid citrate dextrose (0·085 mol Na_3-citrate, 0·065 mol H_3-citrate, 2% (w/v) Dextran T70 (Pharmacia)), placed in disposable plastic tubes and centrifuged (20 min; 500 g). The supernatant, the buffy coat and the upper portion of the erythrocyte sediment were removed.

The remaining erythrocytes and PMN containing sediment was gently mixed with 1–1·5 volumes of 2·5% gelatine (Merck) in 0·9% NaCl. The cell suspension was allowed to sediment in a water-bath that 37°C for 30 min. The PMN containing supernatant above the erythrocytes was carefully removed and centrifuged at 20°C (10 min; 400g). Contaminating erythrocytes were lysed with 0·85% (w/v) NH_4Cl in distilled water. The PMN-suspension was centrifuged at 4°C (10 min; 150 g) and washed twice with cold medium TC 199. The final cell preparation contained more than 90% neutrophils with a viability of greater than 95% as assessed by trypan blue exclusion.

Chemotaxis assay

Fresh or heat treated (30 min, 56°C) serum was diluted with medium TC 199 (1:2) and placed in the lower compartment (0·5 ml) of a modified Boyden chamber of the blind well type. The compartments of the chamber were separated by a membrane filter (mixed cellulose esters; pore size 3·0 μm; Millipore).

The upper compartments were filled with 0·5 ml of the PMN-suspension (3×10^6 cells/ml). The chambers were incubated for 3 h at 37°C. Thereafter the filters were removed, fixed with methanol, stained with haematoxylin, cleared with xylene and mounted on glass slides. All cells present on the lowermost plane of the filter were counted (magnification 500 ×). Ten randomly selected fields were counted and the average number of cells per high power field (HPF) was calculated and expressed as the average of duplicate filters. Casein ('Hammarsten', Merck, 4 mg/ml TC 199) was used as a standard chemoattractant for every PMN preparation; the range of cells per HPF was 20–50. The chemotactic activity of the specimens under study was expressed as the percentage of chemotactic activity of PMN against casein (=relative chemotactic activity, RCA) according to the formula:

$$\mathrm{RCA} = \frac{\text{mean number of PMNs per high power field}}{\text{mean of casein control per high power field.}} \times 100$$

Medium TC 199 alone was used as a negative control.

Preincubation of sera

In order to investigate the ability of PMNs to generate chemotactic activity in autologous serum while migrating through the filters, PMNs from healthy controls were placed in the upper compartments of chemotaxis chambers ($1{\cdot}5 \times 10^6$ cells in 0·5 ml TC 199). The lower compartments contained 0·5 ml autologous serum diluted 1:2 with TC 199. As control 0·5 ml TC 199 without cells was used (upper compartment). A filter (pore size 3·0 μm, Millipore) separated both compartments. After incubation the contents of the lower compartment were heat inactivated and stored at minus 70°C. These sera were then tested for chemotactic activity as described. Further, 1:2 diluted serum (0·5 ml) was incubated together with chemotaxis filters for 50, 90 and 180 min at 37°C in 12 ml plastic tubes. Thereafter the diluted serum was heat inactivated and stored at −70°C until used for chemotaxis.

TABLE 1. PMN chemotaxis with autologous serum

					Heat inactivation		
PMN	Serum	No. of experiments	RCA* (%)	P†	No. of experiments	RCA* (%)	P†
Control	Control	38	152·0 (142–210)	0·001	23	89·5 (48–183)	0·01
Psoriasis	Psoriasis	43	332·5 (242–422)		24	168·5 (81–272)	

* The relative chemotactic activity (RCA) is calculated as the percentage of the control with casein as chemoattractant. The number of PMNs counted per control filter (casein) was between 20 and 50/HPF. The figures for RCA represent the means with 95% confidence interval. The ranges are given in parentheses.

† Level of significance (U-test of Mann & Whitney).

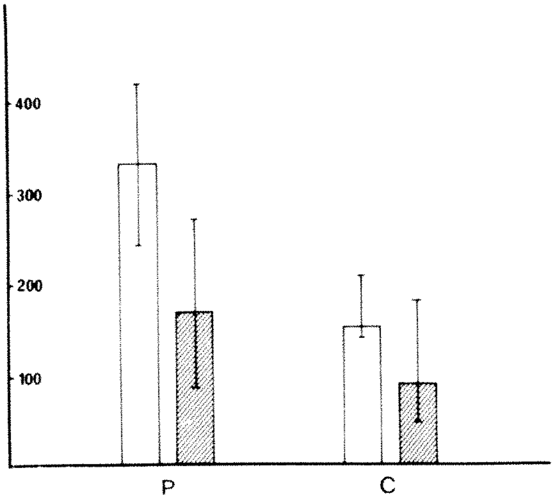

FIGURE 1. PMN chemotaxis using autologous sera as chemoattractants. The relative chemotactic activity (ordinate) is calculated as the percentage of the control chemotaxis assays using casein. Note increased chemotactic activity in the autologous system in psorasis (P) as compared to non-psoriatic controls (C). Differences are still present after heat inactivation (30 min, 56°C) of the sera (shaded columns). Vertical bars: 95% confidence intervals.

RESULTS

PMN chemotaxis against autologous serum

In the autologous chemotaxis assay (psoriatic PMN versus psoriatic serum, normal PMN versus normal serum) an increased chemotactic activity as compared to the controls was present (Table 1, Fig. 1). While in psoriasis the median of the relative chemotactic activity was 332·5 the controls showed only half this value (Table 1). This difference is highly significant. A similar difference between psoriatic and non-psoriatic chemotaxis was seen after heat inactivation of the serum (Table 1). As can be seen in the table, the RCA was reduced by nearly 50% in all assays when the sera were heat inactivated. However the difference ($P<0{\cdot}01$) between the psoriatic and non-psoriatic system was still present.

Effects of non-psoriatic PMNs or sera

For further analysis of this abnormal chemotaxis response in psoriasis we separately compared the chemotactic activity of PMNs and sera with the autologous systems (psoriasis, controls). As before, the experiments were carried out with or without heat inactivation of the sera. As can be seen in Table 2 the enhanced chemotaxis of the autologous psoriatic system was lost when either PMNs or sera from non-psoriatic control persons were tested. Whereas the RCA values for the autologous psoriasis system were quite similar in the two experimental groups (261·5; 275·0, Table 2, A and B) control PMNs responded weakly to psoriatic serum (Table 2A). Psoriatic leukocytes responded to normal serum less actively than to psoriatic serum. This difference was lost after heat inactivation (Table 2B).

Compared to autologous control assays the migratory response of psoriatic PMNs was clearly enhanced and still present when the normal sera were heat inactivated (Table 3A). On the other hand non-psoriatic PMNs were unable to discriminate between the two types of sera (Table 3B)

TABLE 2. Comparison of autologous psoriatic PMNs and sera with non-psoriatic cells and sera

						Heat-inactivation		
Group	PMN	Serum	No. of experiments	RCA* (%)	P†	No. of experiments	RCA* (%)	P†
A	Psoriasis	Psoriasis	26	261·5 (215–433)	0·001	10	118·0 (71–402)	0·05
	Control	Psoriasis		150·0 (122–229)			80·5 (6–113)	
B	Psoriasis	Psoriasis	22	275·0 (207–382)	0·001	9	118·0 (71–403)	n.s.
	Psoriasis	Control		190·8 (127–265)			120·0 (43–170)	

* Median with 95% confidence interval.

† Level of significance (Wilcoxon-test).

n.s. = Not significant.

TABLE 3. PMN chemotaxis in psoriasis: comparison of autologous controls with heterologous PMNs and sera

						Heat inactivation		
Group	PMN	Serum	No. of experi-ments	RCA* (%)	P†	No. of experi-ments	RCA* (%)	P†
A	Control	Control	16	142·5 (102–203)	0·05	11	63·0 (21–135)	0·05
	Psoriasis	Control		172·5 (125–265)			120·0 (42–211)	
B	Control	Control	31	146·5 (131–188)	n.s.	21	69·0 (41–123)	n.s.
	Control	Psoriasis		147·0 (110–202)			56·0 (35·5–113)	

* Median with 95% confidence interval.
† Level of significance (Wilcoxon-test).
n.s. = Not significant.

The autologous psoriatic system (psoriatic PMNs versus psoriatic serum) showed the strongest enhancement of chemotactic activity. In addition the differences between normal and psoriatic PMNs confronted with normal sera were significant demonstrating an increased migratory activity of psoriatic PMNs.

Serum-activation by PMNs

As can be seen in Table 4 pre-exposure of diluted serum in chemotaxis chambers to migrating PMNs enhances the chemotactic activity of the serum. Cells confronted with activated sera showed more active migration (RCA = 243·7) as compared to sera exposed to filters only (RCA = 152·4). Attempts to influence the quality of serum as a chemoattractant by incubation with chemotaxis filters failed to show any effects (Table 5). The RCA data obtained by different lengths of incubation are close to control values.

TABLE 4. Effects of migrating PMNs upon the chemotactic activity of serum

	No. of experiments	Migrating PMNs	Filters only
RCA (%)	3	243·7 ± 32.0	152·4 ± 12.5

TABLE 5. Effects of chemotaxis filters upon the chemotactic activity of serum

	Incubation time (min)			
	50	90	180	control
RCA (%)	40·3 ± 13·0	52·8 ± 10·0	39·8 ± 7·8	55·0 ± 13·4

DISCUSSION

In this study it is shown that PMNs from patients with psoriasis, when confronted with autologous serum, demonstrate a greatly increased chemotactic response. As a method for the assessment of chemotactic activity the Boyden chamber is known to generate rather large standard deviations due to technical variability. We therefore used casein as an internal standard which allows better normalization of the data.

The enhancement of chemotaxis was absent when PMNs from non-psoriatic controls were confronted with serum from patients with psoriasis. Also, heat inactivation which consistently caused a reduction of RCA by approx. 50% revealed no enhancement of serum alone (Table 2). On the other hand there was a moderately enhanced RCA of psoriatic PMNs confronted with control sera (Table 3). Furthermore heat inactivation of normal serum (Table 3) clearly demonstrated the PMN-related enhancement of RCA.

These data suggest that in the psoriatic system the interaction of PMNs with serum generates chemotaxis enhancing components. This is substantiated by our experiments (Table 4) in which serum was pre-exposed to migrating PMNs demonstrating that these cells are able to activate serum. The involvement of neutrophils in generating-chemotactic factors from serum has been described and has been reviewed recently (Wright & Gallin, 1978). In rabbits as well as in man PMNs can cleave the complement component C5 thereby generating C5a. This is a potent chemotactic agent (Snyderman *et al.*, 1969) and in human neutrophils the existence of receptors for C5a has been demonstrated (Chenoweth & Hugli, 1978). Activation of C5a occurs by the secretion of the contents of the specific (secondary) neutrophil granules, which is significantly enhanced when neutrophils adhere non-specifically to surfaces or migrate across cellulose hydrate filters during chemotaxis (Wright & Gallin, 1979).

On the other hand, by measuring phagocytosis, NBT reduction as well as chemotaxis, Krueger *et al.* (1978a) found no abnormality of PMN function *in vitro* in psoriasis. For the chemotaxis assays *E. coli* filtrates were used as chemoattractants. By measuring monocyte chemotaxis against zymosan-activated serum Krueger *et al.* (1978b) subsequently found a 200% increase of chemotactic complement factors in psoriatic subjects. Zymosan activation of serum results in the production of C5 fragments. These observations appear to support our present results. Thus it may follow that in psoriasis increased chemoattractant levels are generated *in situ*, by more active PMNs and by the interaction with autologous serum. Attempts will be made to further clarify these points.

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Herpes gestationis and bullous pemphigoid: a disease spectrum

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SUMMARY

A patient with herpes gestationis has persisted with clinically active disease for 8 years post-partum. She also had Graves' disease, alopecia totalis and ulcerative colitis. The case demonstrates the close overlap between herpes gestationis and bullous pemphigoid.

One of the clinical hallmarks of herpes gestationis (HG) is that following its evolution in pregnancy or the puerperium, it invariably resolves within a few months (Russell & Thorne, 1957). Subsequently, it is likely to recur with succeeding pregnancies and it may be provoked by progesterone oral contraceptives (Lynch & Albrecht, 1966). HG, persisting for up to 28 months post-partum, has been described (Carruthers, Black & Ramnarain, 1977), but the persistence of clinically active disease for 8 years, in the absence of further pregnancies or oral contraceptives, as in our patient, is exceptional.

Also notable in this patient was the association of other 'autoimmune diseases'. Therefore we are describing this case in detail.

CASE REPORT

Mrs P.P., aged 31 years, had her first and only pregnancy at the age of 23 years. In the final 3 weeks of gestation she developed an intensely pruritic rash on the extensor surfaces of her arms and legs. Initially, there were urticated erythematous lesions resembling erythema multiforme and some excoriations; but 2 days post-partum she developed widespread large bullae which were most prominent on the extremities (Figs 1 and 2). There was also some involvement of the buccal mucosa with shallow ulceration.

She had a full term pregnancy with a normal delivery of a healthy female infant who was small for dates, weighing 1·58 kg. The infant's skin was normal.

There was no improvement in the bullous eruption in the puerperium and subsequently the

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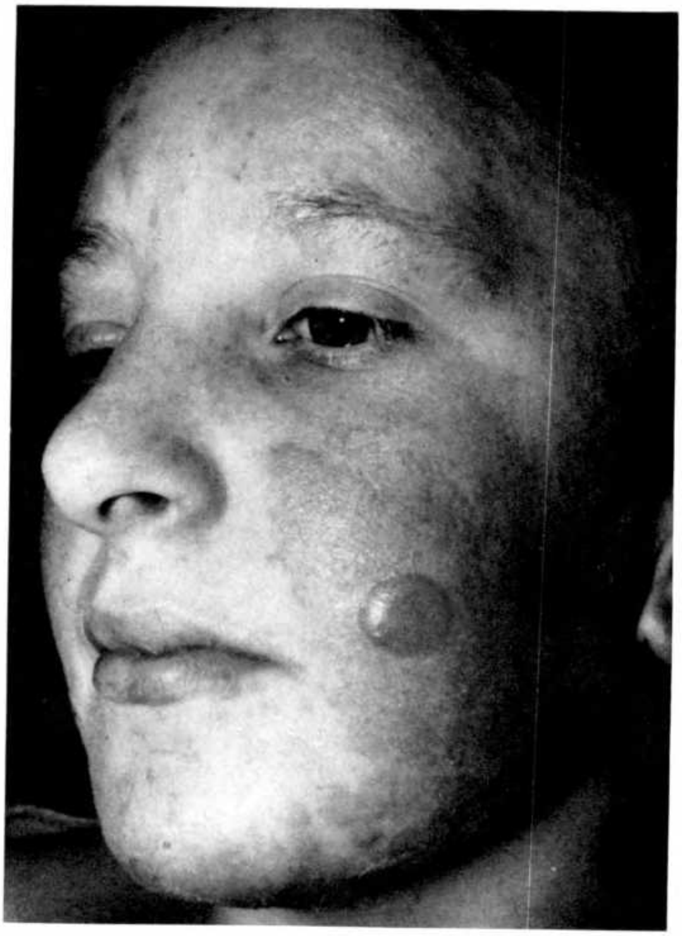

FIGURE 1. Herpes gestationis, initial appearance on the face with a large tense blister on an erythematous base (1971).

disease has remained active over the past 8 years. During the first post-partum year she continued to develop widespread bullae despite treatment with prednisolone 50 mg daily. In the second year of her disease its activity decreased such that it was possible to prevent blistering with prednisolone 25 mg daily and azathioprine 100 mg daily. There were occasional recurrences of blistering, unrelated to menstruation, which were controlled by temporary increases of her steroid dosage. On two occasions she was given dapsone and once sulphapyridine, but these drugs had to be discontinued as they induced a toxic erythema. Her steroid therapy inevitably resulted in Cushingoid changes; her weight increased from 47 kg to 70 kg, and she developed marked striae and a steroid myopathy.

The morphology of her lesions gradually altered during the course of her disease. Initially, she had large bullae on the extremities and face, but over the past 5 years the predominant lesions have been palmo-plantar vesicles and lichenification (Fig. 3) with only occasional bullae on the trunk. She has had no oral contraceptives during this time.

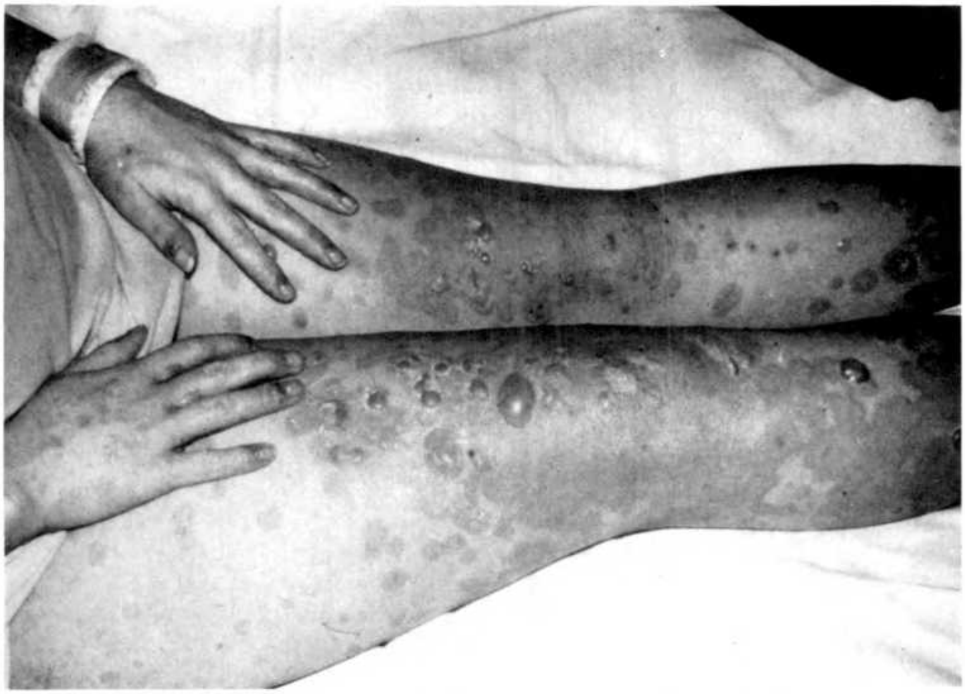

FIGURE 2. Large blisters with urticated erythema on the legs and hands (1971).

Histology

Biopsy of an early bullous lesion showed a sub-epidermal bulla associated with a dermal infiltrate consisting predominantly of lymphocytes and eosinophils (Fig. 4). A biopsy taken adjacent to a vesicular area on the sole of the foot showed some oedema of the epidermis with foci of spongiosis. There was some oedema in the papillary dermis, and around the blood vessels there was a predominantly lymphohistiocytic infiltrate with some eosinophils.

Immunofluorescence

Lesional skin and serum were initially examined by immunofluorescence elsewhere, and 1 year after the onset of her disease both were reported as negative. However, at this time staining for C3 was not included in either the direct or indirect technique. Our laboratory first received material for immunofluorescence in 1976 which was 5 years after the onset of the disease. Direct immunofluorescence of bullous skin was positive at the basement membrane zone for IgG and C3. Indirect immunofluorescence was positive for C3 (titre 1 in 16) and IgG (titre 1 in 640). Indirect complement immunofluorescence was performed by the method of Carruthers, Black & Ramnarain (1977). Over the past 3 years the direct immunofluorescence of both the bullous lesions and the palmo-plantar eczematous appearing lesions has remained positive (similarly the indirect immunofluorescence of her serum has remained positive with a notable increase in the titres). In December 1979 the indirect immunofluorescence C3 titre was 1 in 64 and IgG titre 1 in 2560.

In addition to her bullous disease she has had ulcerative colitis, alopecia totalis and Graves' disease. Two years prior to her only pregnancy she had an episode of bloody diarrhoea. Proctosigmoidoscopy showed shallow bleeding ulcers in the upper rectum. This acute episode settled without

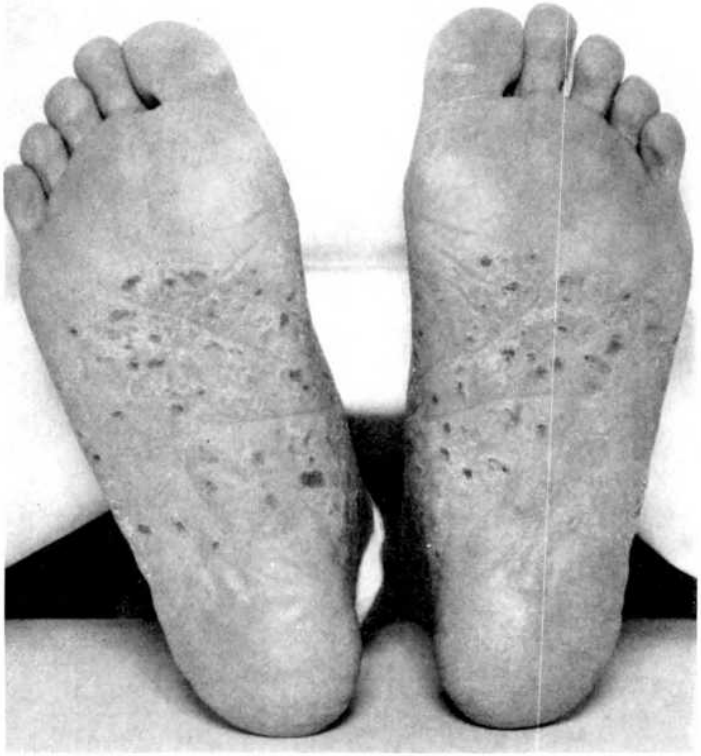

FIGURE 3. Eczematous apperance on the plantar surface of the feet (1978).

administering systemic steroids. Subsequently, she has had intermittent mild abdominal pain with some rectal bleeding. The colitis was re-investigated in October 1978, which was 9 years after the onset of her symptoms. A barium enema, rectal biopsy and sigmoidoscopy all confirmed ulcerative colitis.

Her alopecia areata developed at the age of 22 years. Initially this was patchy, but later became total. Two years after the onset of hair loss there was complete re-growth, and since then there has been no recurrence.

Graves' disease was diagnosed in October 1978, when it was noted that she had exophthalmos and lid lag. There was a small firm goitre with a loud systolic bruit. The plasma thyroxine level was elevated at 300 nmol/l (normal range 62–133 nmol/l). Her thyrotoxicosis was attributed to Graves' disease as she had thyroid cytoplasmic auto-antibodies (titre 1 in 400) and her thyroid scan showed a generalized increased uptake. She had a partial thyroidectomy, the thyroid histology being consistent with autoimmune thyrotoxicosis. In 1979 it was noted that she had gastric parietal cell antibodies; however, there was no evidence of pernicious anaemia as her serum vitamin B12 was normal and there were no megaloblasts in her bone narrow. Her antinuclear factor was negative and there were no smooth muscle or antimitochondrial antibodies. There was no family history of atopy, colitis, skin disease or thyroid disease.

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Immunopathological studies on rosacea

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SUMMARY

Seven patients with papulo-pustular rosacea were investigated with immunofluorescence (IF) techniques. Indirect IF has been performed also with antibodies eluted from circulating lymphoid cells. Besides confirming the data in the literature on the positivity of the basal zone, anticollagen antibodies were found, and eluted antinuclear antibodies were detected against nuclei of cells in the epidermis and dermis, namely, scattered dermal, endothelial and eccrine duct cells.

Fluorescent patterns of immunoglobulin and complement staining at the dermo-epidermal junction in rosacea have been described by several authors (Baart de la Faille & Baart de la Faille-Kuyper, 1969; Salö, 1970; Jablonska, Chorzelski & Maciejowska, 1970; Gajewska, 1975; Boorsma, Nieboer & Kalsbeek, 1975) and denied by Abell, Black & Marks (1974). The contrasting opinions might be due, at least in part, to the different criteria by which patients have been selected. In particular, patients with erythrosis and telangiectases can be confused with systemic lupus erythematosus (SLE) patients more easily than one may imagine. In Salö's (1970) series ten patients out of twenty-seven had general symptoms which could have strongly suggested SLE.

We have repeated and extended immunological observations on patients affected by rosacea in which papulo-pustules were clinically predominant and a granulomatous pattern was found histologically.

MATERIALS AND METHODS

Seven untreated patients (four males and three females), varying from 36 to 76 years of age, were studied. These patients had complained of papulo-pustular rosacea of the face for between 1 and 4 years.

The diagnosis was based on clinical and histological criteria: a granulomatous infiltrate was found in all cases around follicles and deep in the dermis. Demodex folliculorum was invariably found in KOH scrapings. No histological signs suggesting LE. were present in our patients.

Six normal subjects (four males, two females) aged between 30 and 42 years served as controls for serological tests and for tests on the eluates from circulating lymphoid cells.

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Chemicals and Reagents

Chemicals and reagents have been detailed by Cormane, Hammerlinck & Nunzi (1979).

Lymphoid cell separation and elution of antibodies from circulating lymphoid cells

Techniques have been described by Cormane *et al.* (1979).

Substrates

Biopsy specimens were obtained under local anaesthesia (2% carbocaine) with a 3 mm punch from: (A) Area affected by rosacea (malar area). (B) Clinically normal area but light exposed (lateral area of neck). (C) Clinically normal area non-light exposed (deltoid area).

As control substrates we have used clinically and histologically normal specimens obtained from the malar area.

For the immunofluorescence (IF) studies, *in vitro* rat tongue and rat liver were also used as substrates.

Immunofluorescence studies

Preparation of the skin sections. Biopsy specimens were prepared according to the techniques described by Cormane, Szabo & Hauge (1970). Sections were cut at 4 μm with a cryostat (International Equipment Co, Washington, U.S.A.) and dried on microscopic slides for 30 min. Next, they were washed in PBS (pH 7·2) for 30 min and then incubated with conjugates in a moist chamber for 30 min. Finally, they were washed twice for 30 min in PBS (pH 7·2).

In vivo IF studies of the skin sections. For the demonstration of tissue- and cell-bound Ig (i.e. IgG, IgA, IgM, IgD and IgE), C3c, Fab (IgG) and Fc (IgG) in the skin biopsies, the conjugates were used as shown in Table 1.

It is worth noting that cytoplasmic fluorescence of ancillary cells in skin sections is observed only if specific agglutinin antisera are used (Table 1).

In vitro IF studies of the skin sections. For the detection of circulating antibodies in patients, the indirect IF method was applied (IIF). Clinically and histologically involved areas, uninvolved light exposed areas, uninvolved non-light exposed areas, rat tongue and rat liver were used as substrates.

For detection of antibodies in the eluates, skin sections were first incubated with the eluate of isolated circulating lymphoid cells from the same patient and subsequently with FITC labelled antisera (Table 1). Patients 1 and 2 did not participate in studies using this technique.

In vitro complement fixation test (IIF). Indirect complement staining was carried out for the detection of complement fixing antibodies in the eluates on unfixed sections of rat tongue, according to the method described by Jordon, Nordby & Milstein (1961) and detailed by Cormane *et al.* (1979).

Eosinophilic granulocyte studies. The FITC (±1 μg/ml) staining was used for detecting eosinophils (Berretty & Cormane, 1978).

Microscopy. A Leitz Orthoplan microscope with a Xenon WBO-75 lamp and a Leitz vertical illuminator were used. FITC-fluorescence was detected using the filter combination BG 38 (4 mm), KP 490 (2 mm) and secondary filter OG 515 (3 mm). Leitz water immersion objectives (×22, ×50 and ×90) were used in all cases.

TABLE 1. Characteristics of FITC-protein conjugates

Conjugates	Batch no.	F/P ratio (μg/mg)	Molar ratio F/P × 0·411	Antibody unitage	Working dilution
Rabbit antihuman* IgG/FITC	4-572	2·7	1·11	2	1/40
Rabbit antihuman† IgA/FITC	KH 14-15-FO1	2·5	1·02	1	1/40
Rabbit antihuman† IgM/FITC	KH 15-18-FO1	2·3	0·94	4	1/40
Rabbit antihuman‡ IgD/FITC	KH 20-07-AO3	1·2	0·49	8	1/40
Swine antihuman* IgE/FITC	7701	0·89	0·36	n.d.	1/40
Rabbit antihuman‡ C3c/FITC	KH 41-9-PO1	2·8	1·17	2	1/40
Goat antihuman(CH3)* IgG(Fc)/FITC	2–372	2·6	1·06	n.d.	1/40
Goat antihuman* IgG(Fab$_2$)/ FITC	9-275	5·6	2·3	n.d.	1/40

* Nordic Immunological Laboratories, Tilburg, The Netherlands.

† Central Laboratory of the Red Cross Blood Transfusion Services, Amsterdam.

‡ The unconjugated antisera were obtained from the Central Laboratory of the Red Cross Blood Transfusion Services, Amsterdam, and were labelled by S.S. Asghar PhD (Biochemistry Laboratory, Department of Dermatology, University of Amsterdam).

n.d. = Not done.

GaHu/Fab/FITC, batch 9-275, was obtained by conjugation with FITC of the isolated and purified IgG fraction of an antiserum raised by immunization with Fab of normal human serum IgG. The conjugate reacted with gammaFab of both light chain types. Potency and specificity of the conjugate were tested by direct cytoplasmic staining of normal and monoclonal human bone marrow plasmocytes and peripheral blood lymphocytes, and by surface staining of vital normal and pathological peripheral blood lymphocytes. FITC content of conjugates was determined by the method of McKinney, Pearce & Spillane (1964) using fluorescein diacetate (FDA) as reference standard. Protein content was measured by the method of Lowry *et al.* (1951). Antibody unitage conjugate was determined in a standard gel diffusion test using 1 mg/ml respective antigens except in one case of FITC/C3c conjugate where normal serum was used as a source of antigen against doubling dilutions of conjugates as described by Beutner, Sepulveda & Barnett (1968). A 1 mg/ml solution of human IgG, IgM, IgA and IgD contained 6·6, 129, 30·5 and 100 antigen units respectively in relation to international reference serum. Staining properties and specificity of the class-specific conjugates were tested in IF in normal and monoclonal human bone marrow plasmocytes.

Specificity tests. For the IIF method with the eluates, duplicate sections of those showing a positive reaction with the eluates were treated directly with the supernatant stored as control and subsequently with the conjugates mentioned in Table 1. In all sections treated as such, negative results were obtained.

RESULTS

Detection of immunoglobulin deposit in vivo

The basal zone (BZ) was positive in five patients out of seven in the involved skin (Area A), in two patients out of seven in the uninvolved light-exposed skin (Area B) and in one patient out of seven in the uninvolved non-light exposed skin (Area C).

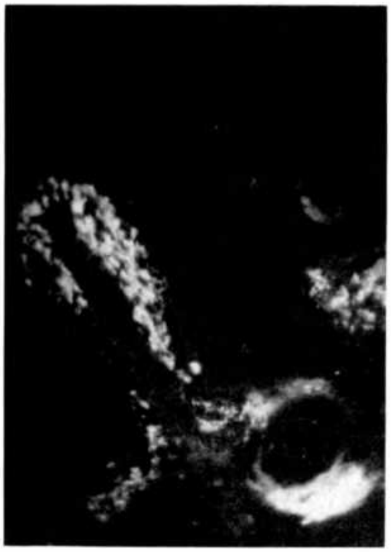

FIGURE 1. Cryostat section of involved skin of Patient 6 (DIF). Note altered collagen fibres located in upper dermis reactive to RA Hu IgM/FITC.

In addition anticollagen-antibodies were found in six out of seven patients in Area A, four out of seven in Area B and one out of seven in Area C (Fig. 1).

Positive cells were found scattered in the dermis in five out of seven in Area A, two out of seven in Area B and three out of seven in Area C.

A large number of eosinophils were found with FITC staining in four out of seven patients (1, 5, 6, 7) in Area A, five out of seven patients (1, 2, 3, 4, 5) in Area B and three out of seven patients (5, 6, 7) in Area C.

Details concerning the classes of immunoglobulins and complement fractions are presented in Table 2.

Demonstration of circulating and lymphocyte-eluted antitissue antibodies

(1) Circulating antibodies against BZ were found in two patients (1 and 3) in Area A appertaining to the classes IgA and IgG and in Area B in one patient (3) with IgG determinants.

TABLE 2. Direct immunofluorescence studies

Targets	Patient/Sex/age	Substrates		
		Involved skin	Uninvolved skin	
			Light exposed	Non-light exposed
Basal zone	1/M/76	Fab	—	—
	2/F/65	—	—	—
	3/F/47	IgM, Fc	—	—
	4/F/70	IgG, IgA, Fc, Fab	IgG, IgA, IgM, Fab	—
	5/M/36	IgG, IgM, Fc	—	—
	6/M/37	IgA, IgM	IgG, IgM	IgM
	7/M/47	—	—	—
Collagen	1/M/76	IgG	—	—
	2/F/65	IgM	IgM	IgM
	3/F/47	IgG, IgA, IgM	IgA, IgM	—
	4/F/70	IgG, IgM	IgG, Fab, Fc	—
	5/M/35	IgG, IgM	—	—
	6/M/37	IgG, IgA, IgM	IgG, IgM	—
	7/M/47	—	—	—
Cells in the dermis	1/M/76	IgA, IgM, IgE, C_3	IgM	—
	2/F/65	—	—	—
	3/F/47	IgA	—	—
	4/F/70	—	—	—
	5/M/36	IgM, IgE, C_3, Fab	IgM, IgD	IgM
	6/M/37	IgE, Fab	—	IgM, IgD, IgE, C_3, Fab
	7/M/47	IgM, IgE, Fab	—	IgM, C_3

— = negative

Circulating antibodies against collagen of the classes IgG and IgM were found in three patients (1, 3, 5) in Area B.

Circulating ANA antibodies (IgD, IgM and IgE) with homogeneous pattern were found with serum dilution 1/40 only in two patients (1 and 3) in Area A.

In a single case the mite *Demodex folliculorum* was found in an infundibulum and reacted with serum IgG (Fig. 2).

(2) Antibodies eluted from circulating lymphoid cells were found against nuclei (homogeneous pattern) of epidermal cells in two patients in Areas B and C, appertaining to the IgM class.

Eluted antibodies against nuclei of various cells (dermal cells, endothelial cells and eccrine duct cells) were found in the dermis in all patients and in all areas, mainly of the IgM and IgE classes (Table 3; Fig. 3a and b).

(3) Antibodies eluted from circulating lymphoid cells obtained from normal controls were always negative when challenged with normal control substrates. On the contrary, antinuclear activity (homogeneous pattern) of the IgM and IgE classes was revealed against epidermal and especially scattered dermal cells when antibodies from lymphoid cells of controls were challenged with rosacea substrates.

Antibodies eluted from circulating lymphoid cells obtained from rosacea patients did not react at all when challenged with normal substrates.

(4) None of the eluted antibodies showed complement binding '*in vitro*' using rat tongue as substrate.

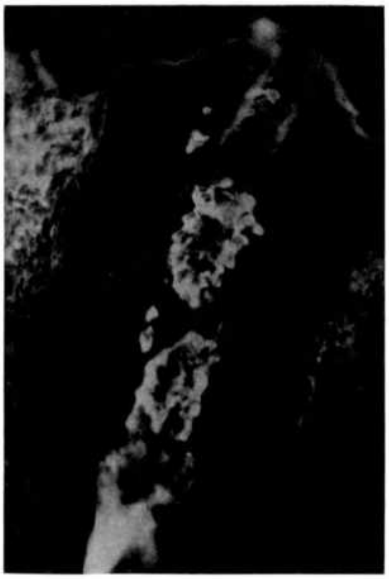

FIGURE 2. Cryostat section of involved skin of Patient 4 (IIF). The section was first incubated with serum of patient (dilution 1/40) and subsequently with RA Hu IgG/FITC. Note IgG present in the serum reactive to the surface antigens of *Demodex folliculorum* in an infundibulum.

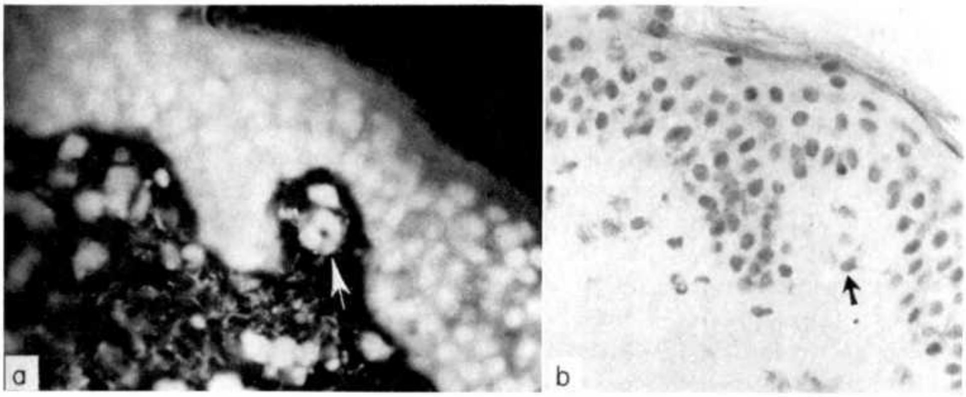

FIGURE 3. (a) Cryostat section of uninvolved non-light exposed skin of Patient 6 (IIF). The section was incubated with eluate of isolated circulating lymphocytes of the patient and subsequently with RA Hu IgM/FITC. Note IgM present in the eluate reactive to epidermal cells, scattered dermal cells and endothelial cells of a capillary loop (arrow). (b) The same section as (a) stained with haematoxylin eosin.

TABLE 3. ANA eluted from circulating lymphoid cells

		Substrates		
Target nuclei	Patient/sex/age	Involved skin	Uninvolved skin	
			Light exposed	Non-light exposed
Epidermal cells	1/M/76	n.d.	n.d.	n.d.
	2/F/65	n.d.	n.d.	n.d.
	3/F/47	—	IgM	IgM
	4/F/70	—	—	—
	5/M/36	—	—	—
	6/M/37	—	IgM	IgM
	7/M/47	—	—	—
Cells in the dermis*	1/M/76	n.d.	n.d.	n.d.
	2/F/65	n.d.	n.d.	n.d.
	3/F/46	IgM, IgE	IgD, IgE	IgD, IgE
	4/F/70	IgA, IgM, IgE	IgD, IgE	IgM, IgD, IgE
	5/M/36	IgE	IgE	IgM, IgD, IgE
	6/M/37	IgM, IgE	IgM, IgE	IgM, IgE
	7/M/47	IgM	IgM, IgE	IgM, IgD, IgE

n.d. = Not done.
— = Negative.
* Nuclei of scattered cells, endothelial cells and eccrine duct cells.

The ANA antibodies detected in the serum and in the eluates are species-specific since they do not react with liver and rat tongue.

DISCUSSION

As mentioned in the introduction, the diagnosis of rosacea may present some difficulty. In particular, we took care to exclude SLE in our patients according to clinical and histological criteria. A problem, however, arose in considering demodicidosis in the differential diagnosis. In fact, the criteria for distinguishing the two clinical manifestations are surprisingly poor in the literature and can be summarized in Ayres' statement (Ayres & Ayres, 1961) that papulo-pustular rosacea has smaller pustules and a more oily appearance than demodicidosis. Even though heavy infestation with the mites is certainly accompanied by dryness of the skin and by a particular cretaceous scaling appearance, it is impossible, in our opinion, to trace a definite borderline between a papulo-pustular rosacea with Demodex and a rosacea-like demodicidosis.

Having excluded cases of heavy infestation with the mite, our patients could be defined as papulo-pustular rosacea with Demodex.

In vivo studies confirm data from the literature. According to Jablonska *et al.* (1970) all conditions with telangiectases can cause positivity of the basal zone (BZ); Burnham & Fine (1971) advanced the hypothesis that BZ positivity is false and that it could be due to the oedema and the infiltrate which, by compressing the dermal components of the papillae, would produce a 'band-like effect'.

We suggest that the BZ positivity may be due to antibodies directed against altered collagen of Type 4, which is present in the sub-basal lamina in contact with the corium.

Such collagen alteration may be due to exposure to light. Actinic elastosis has already been stressed by Marks & Harcourt-Webster (1969) as being excessive in rosacea, and may be responsible for the anticollagen-antibodies we found in the papillary dermis and in BZ in rosacea patients. In fact, the fluorescent pattern of BZ appears to be similar to the pattern which has been found in BZ by Yaoita, Foidart & Katz (1978) with antibodies to collagen Type 4.

The presence of eosinophils, already noted by Marks & Harcourt-Webster (1969), is not so easy to explain. They might be related to the mites *Demodex folliculorum* which were present in various numbers in the hair follicles and sebaceous glands in all our patients.

Results obtained from elution studies appear to be more interesting. Anti-DNA antibodies without affinity for complement from circulating lymphoid cells from normal people as well as from rosacea patients react only to rosacea substrates. This finding strongly suggest that a change has occurred in the skin rather than in the immunocompetent system. The incapacity of normal people's skin to react to this kind of antibody present on lymphoid cells in every subject may be explained as follows: (1) Thymine dimers are formed by UV and are not removed for some reasons (defect in the DNA-repair system?) peculiar to rosacea patients. Consequently, anti-UV DNA antibodies are raised and react to nuclear structures in the epidermis and dermis of rosacea patients. Anti-UV DNA antibodies are also found in normal people in an insignificant amount (Davis, 1977) and cannot react to their target because of their immediate removal. When challenged with rosacea substrate, however, anti-UV DNA antibodies of normal people react. (2) Antibodies directed against nuclei of *Demodex folliculorum* cells are present which cross-react to nuclei of epidermal and dermal cells. (3) Immunoglobulins penetrate the nucleus of dermal and epidermal cells, as suggested by Hannestad (1979) and Galoppin & Saurat (1980). Such penetration might be favoured by topical corticosteroids used for various periods of time by all our patients.

Antibodies eluted against endothelial cells of small vessels (Cormane, Hammerlinck & Nunzi, 1979) in the upper dermis may be related to the clinical and pathological evidence of vasculitis, which has been described in rosacea (Marks, 1968) possibly due to environmental stimuli (Marks, 1973), especially light (Miescher, 1957).

In conclusion, our findings may help to explain some clinical events in the natural history of the disease.

First of all anticollagen activity may account for the occurrence of the telangiectasia, due to damage to the vessel wall and/or the perivascular tissue. Telangiectasia may also occur as a direct consequence of the immunological injury to the endothelial cells.

Secondly, collagen, nuclei of various dermal cells and *Demodex folliculorum* have been shown to induce an immunological response that may account for the passage from a predominantly functional phase to the inflammatory phase of the disease.

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The immunofluorescence techniques were performed in the laboratory of immunopathology of the Department of Dermatology, University of Amsterdam.

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Case Reports

Secondary localized cutaneous amyloidosis in melanocytic naevi

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SUMMARY

Amyloid deposits were identified within the tumour mass of three melanocytic naevi. Amyloid in this situation is likely to be derived from degenerating naevus cells.

Deposits of amyloid are well known to localize around various neoplastic and degenerative skin lesions. This secondary localized cutaneous amyloidosis has been described in association with basal cell epithelioma (Sumegi, Goreczky & Roth, 1954; Malak & Smith, 1962; Brownstein & Helwig, 1970; Hashimoto & Brownstein, 1973; Weedon & Shand, 1979), fibroepithelioma of Pinkus (Sumegi, 1973), pilomatrixoma (Peterson, 1968), cylindroma (Freudenthal, 1930; Heyl, 1966), seborrhoeic keratosis (Malak & Smith, 1962), actinic keratosis (Hashimoto & King, 1973), Bowen's disease (Brownstein & Helwig, 1970), disseminated superficial actinic porokeratosis (Piamphongsant & Sittapairoachana, 1974) and odontogenic tumours (Meenaghan, Appel & Greene, 1972). Specific reports of the occurrence of amyloid within melanocytic naevi are lacking. We have recently observed three cases of melanocytic naevi incorporating localized amyloid deposits.

MATERIALS AND METHODS

In the course of routine histological examination of melanocytic naevi removed for cosmetic reasons, three lesions were seen showing obvious deposits of a material which appeared amorphous in haematoxylin and eosin stained sections. The patients were two males, aged 19 and 33, and one female aged 33. One of the three lesions was from a sun exposed site.

Paraffin sections of the three lesions were stained with Congo red, crystal violet and thioflavine T, and submitted to routine and polarization light microscopy.

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RESULTS

Two of the lesions were compound melanocytic naevi, while the third was entirely intradermal. On H & E stained sections rounded pink amorphous deposits were situated between naevus cells and appeared as clumps of globular bodies (Fig. 1). The size and localization of the aggregates of amorphous material were similar to naevus cell nests (Fig. 1). In two lesions the amyloid was situated in a subepidermal position, while in the third it occupied a central position within the naevus.

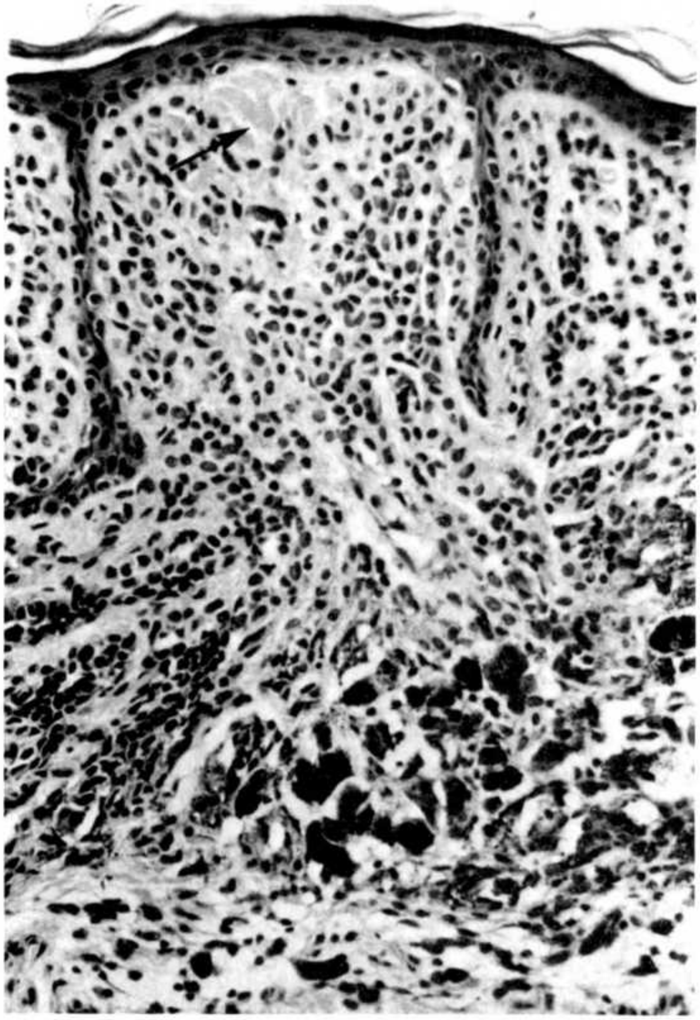

FIGURE 1. Amyloid deposit (↑) in a subepidermal position within a dermal melanocytic naevus (H & E, × 67).

In all cases the amorphous material stained positively for amyloid with the Congo red and crystal violet stains. Polarization microscopy of Congo red stained sections showed characteristic green birefringence. Thioflavine T-stained specimens examined by fluorescence microscopy exhibited yellow fluorescence of the localized deposits confirming their amyloid nature.

DISCUSSION

In primary localized cutaneous amyloidosis it is most likely that the amyloid is derived from degenerating epidermal cells (Black & Wilson Jones, 1971; Black, 1971; Black & Heather, 1972; MacDonald, Black & Ramnarain, 1977; MacDonald & Black, 1977; Kumakiri & Hashimoto, 1979; Hashimoto & Kumakiri, 1979).

Secondary localized cutaneous amyloidosis has been reported principally in lesions of epithelial origin where degeneration of epidermal cells occurs. Weedon & Shand (1979) have emphasized that the occurrence of amyloid in basal cell epitheliomas is more prominent in slowly growing tumours with more numerous apoptotic cells. Fibrils resembling amyloid have been found on ultrastructural examination of cultured cells from basal cell epithelioma (Bleehen, 1975). The microfibrils or tonofilaments of dying tumour cells may be converted to a substance with the physicochemical properties of amyloid (David & Buchner, 1978) including a β pleated sheet x-ray diffraction analysis pattern which is known to be shared by amyloid and keratin (Gueft, 1972).

The occurrence of amyloid within melanocytic naevi and in particular the distribution we have observed suggest that degenerating naevus cells may also contribute to the formation of amyloid. Existing evidence implicates the melanocyte in the pathogenesis of cutaneous amyloidosis. DOPA-positive cells are absent in the epidermal basal layer in the vicinity of amyloid deposits in primary localized cutaneous amyloidosis (Black, 1971) and deposition of amyloid may be found in association with vitiligo (Hashimoto, 1979). Hashimoto & Kumakiri (1979) have reported the development of amyloid-like filaments in keratinocytes adjacent to melanocytes in PUVA-treated patients. Pigment-bearing cells have frequently been found in cutaneous amyloid deposits. Such cells possessing obvious dendritic processes have been interpreted as melanocytes by Westermark (1979). It is possible that polymerization of subcellular proteins of melanocytes may give rise to a substance with the staining properties of amyloid whose biochemical composition may differ from that derived from degeneration of keratinocytes.

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Miliary tuberculosis in association with chronic cutaneous tuberculosis

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SUMMARY

A case of miliary tuberculosis associated with two different forms of cutaneous tuberculosis and a negative Heaf tuberculin test is reported. The value of skin biopsy and culture in helping to establish an early diagnosis is discussed. The unusual finding of a negative Heaf tuberculin test is commented upon.

CASE HISTORY*

A 67-year-old retired farm worker presented to his general practitioner complaining of loss of appetite, a cough productive of yellow sputum of 6 months duration, together with loss of weight of about one stone over the past 2 months. A chest X-ray showed generalised miliary shadowing of the lung fields with additional opacities at the apices; consequently the patient was referred to a chest unit.

Examination on admission to hospital revealed an emaciated man with a pyrexia of 37·5°C. He was found to have a skin condition which he said had been present on his right leg for 8 years and on his chest and left arm for 4 years; he had not sought advice for this.

The skin findings were as follows: on the right leg there was a swelling due to non-pitting oedema with papular changes considered to be compatible with lymphoedema. Extending from mid-thigh to the dorsum of the right foot was a scaly, brownish, atrophic eruption (Fig. 1) and on the lateral aspect of the leg, just above the right knee, there was an ulcer surrounded by an area of marked scarring. On the left side of the chest anteriorly and on the left upper arm there was an area of infiltration of the skin with atrophy and a brownish discolouration. The only other finding of note was a pleomorphic adenoma of the parotid gland.

The blood count, liver function tests and plasma protein estimations were normal apart from a raised alkaline phosphatase of 338 i.u. The Heaf tuberculin test was negative. The Kveim test was also negative.

Skin biopsy samples were taken from four separate sites—the leg ulcer, the right limb and from the chest and upper arm lesions. The histopathological changes were those of tuberculous granulomata

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* This patient was shown at the Dowling Club Meeting in Newcastle upon Tyne on 23rd September 1978.

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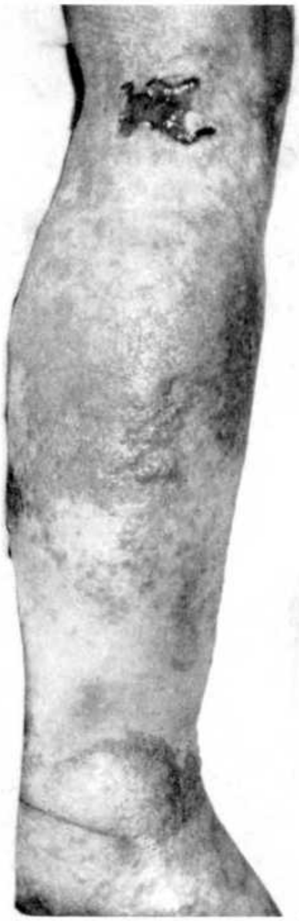

FIGURE 1. Right leg.

mostly situated in the upper dermis and showing occasional small foci of caseation necrosis. Acid fast bacilli were abundant in three out of the four biopsies. Many granulomata contained multinucleated giant cells of Langhans type, close to the epidermis and surrounded by dense lymphocytic and plasmacytic infiltrate (Fig. 2).

The sputum was negative for acid-fast bacilli on direct smear, but culture grew *Mycobacterium tuberculosis var. hominis*, sensitive to the usual anti-tuberculous drugs. A smear from the ulcer showed acid-fast bacilli; culture was negative. The cultures of all the skin samples were positive for *Mycobacterium tuberculosis var. hominis*.

The patient was treated with rifampicin 600 mg each morning and Mynah 250 three tablets each morning (containing a total of 300 mg of isoniazid and 750 mg of ethambutol) for 2 months, followed by Rifinah 300, two tablets each morning (containing a total of 300 mg isoniazid and 600 mg rifampicin) for a further period of 7 months. His original symptoms of cough, anorexia and weight loss

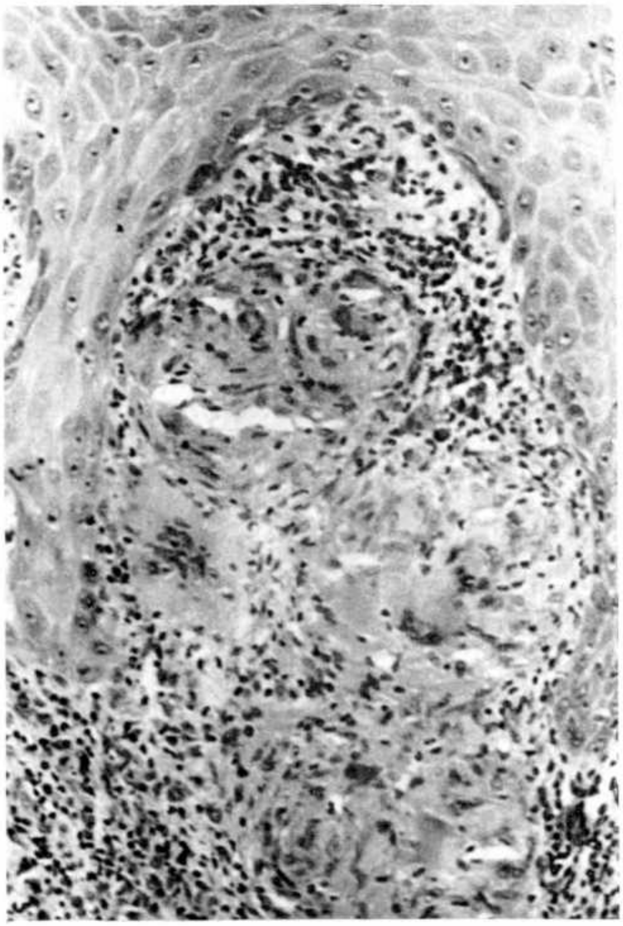

FIGURE 2. Granuloma with Langhans giant cells.

responded rapidly to this therapy. The leg ulcer healed by scarring within one month. The skin lesions steadily improved leaving only residual fine atrophic changes and some pigmentation. The lymphoedema of the leg has persisted. His chest X-ray repeated 6 months after treatment showed some regression of the miliary mottling only. The Heaf tuberculin test subsequently became grade III positive.

DISCUSSION

The clinical appearance of the skin changes on the leg of our patient resembled previous descriptions of lupus vulgaris (Wilkinson, 1979), but the lesions on the chest and upper arm were less usual and clearly a form of disseminated cutaneous tuberculosis was the most likely diagnosis. The histological appearances of both types of skin lesion were very suggestive of cutaneous tuberculosis and this diagnosis was proven by the subsequent positive cultures for *Mycobacterium tuberculosis var. hominis* from all the skin biopsy samples.

The frequency of finding mycobacteria in histological sections of biopsies of cutaneous tuberculosis is variable and it may be difficult to demonstrate them in lupus vulgaris. Cultures of skin biopsies are rarely positive; Horwitz, in his extensive study of lupus vulgaris, reported that only 6% of his biopsies were positive on culture (Horwitz, 1960). Clearly, the findings in this case are unusual in this respect, since in three of our biopsy samples numerous mycobateria were seen in Ziehl-Nielsen staining and subsequently grown in culture from all the skin samples.

A negative tuberculin test is unusual in lupus vulgaris and none of the conditions known to be associated with a negative tuberculin test was present in this case since the patient was not critically ill (*British Medical Journal*, 1970). In this case the negative tuberculin test may have reflected a temporary specific anergy to tuberculin, thereby permitting survival of the mycobacteria in the skin lesions and being associated with his clinical deterioration. Cutaneous tuberculosis is now rare in this country and one of the purposes of this report is to emphasize its continued occurrence. Miliary tuberculosis is rarely seen with cutaneous tuberculosis (Horwitz, 1960) and its association with two different forms of cutaneous tuberculosis presented certain diagnostic difficulty especially in view of the negative direct sputum smear and a negative Heaf tuberculin test. The skin biopsy findings were crucial in establishing a rapid and accurate diagnosis and appropriate therapy could be started with confidence well before the positive cultures were available.

ACKNOWLEDGMENTS

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Lichen scrofulosorum with tuberculous dactylitis

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SUMMARY

A patient with tuberculosis of cervical lymph nodes, lichen scrofulosorum on the trunk and limbs and tuberculous dactylitis of the hands is reported. Both the clinical and histological features of her lichen scrofulosorum conformed to the characteristic pattern described in other recently reported cases and the association with extra-pulmonary tuberculosis was again evident. There have been no other recent reports of tuberculous dactylitis, which was always said to be due to underlying tuberculous bony involvement. In our patient tuberculous dactylitis, which was clinically indistinguishable from previous cases, was found to be due to granulomatous involvement of the overlying skin with no associated bony abnormality.

This is a report of a patient with lichen scrofulosorum and tuberculous dactylitis. Lichen scrofulosorum was a familiar though uncommon clinical entity to dermatologists of the first half of this century, but very few documented cases have been reported since the advent of chemotherapy for tuberculous disease. Some (Ockuly & Montgomery, 1950) may have confused lichen scrofulosorum with cutaneous sarcoidosis, others (Lever, 1967) have denied its existence and even recent textbooks (Wolff, 1979) pay little attention to it. Six reliably documented cases (Smith *et al.*, 1976; Hudson, 1976; Russell-Jones *et al.*, 1979) and the patient presented here suggest that the reappearance of tuberculous disease in this country is occasionally accompanied by the rarer forms of skin involvement, especially lichen scrofulosorum.

Tuberculous dactylitis is yet another rare form of tuberculosis mentioned in old texts. It is inadquately delineated and in the past it has been considered always to be based on associated involvement of bone. No bony changes were present in our patient and the dactylitis was caused by granulomatous involvement of the skin of the affected digits.

CASE REPORT

Miss M.B., a 22-year-old Tanzanian Asian, was admitted to the Royal Free Hospital for investigations of malaise and weight loss of 2 months duration. Her disease started with a sore throat for

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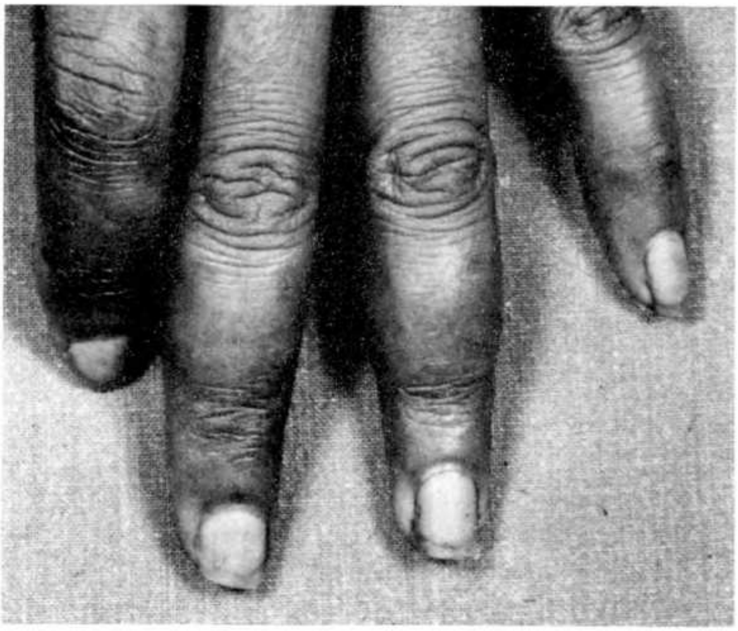

FIGURE 1. Fusiform swelling of the middle portions of the fingers.

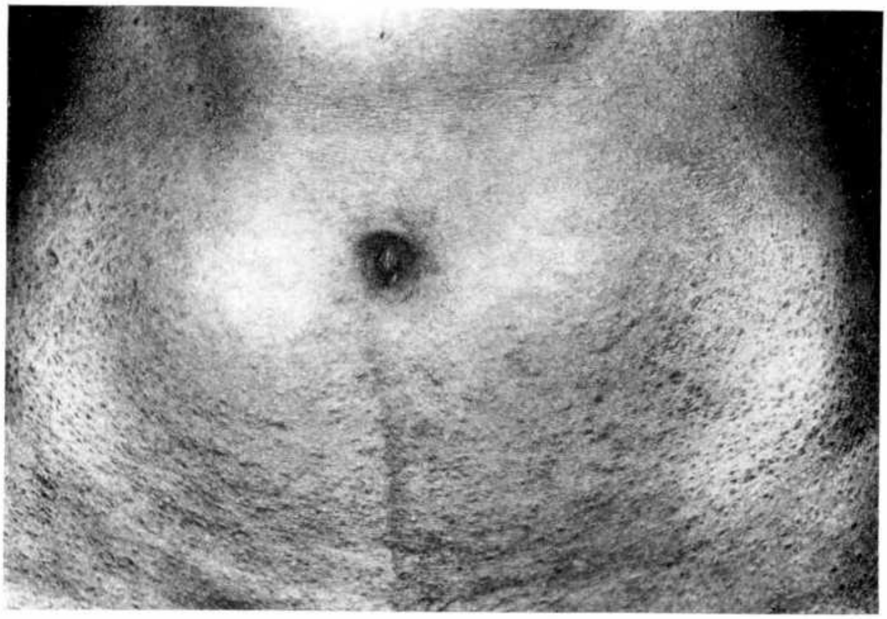

FIGURE 2. Lichenoid papules on the abdomen, characteristic of lichen scrofulosorum.

which she received a week's course of Cephalexin. She had been resident in the United Kingdom for eight months and had no history of contact with tuberculosis.

On examination, she had gross, non-ulcerating, non-tender, bilateral cervical lymphadenopathy, but no enlarged lymph glands elsewhere. Her liver was felt 2 centimetres below the costal margin but her spleen was not palpable. There was doughy, spindle-shaped swelling of the middle phalangeal portions of several fingers (Fig. 1). These almost cylindrical phalangeal enlargements were not tender and the joints were not involved. There was no obvious change in the colour of the skin of the affected fingers. On the chest, abdomen and all limbs, her skin was covered by a fine eruption of lichenoid papules, dark brown-violaceous in colour, made more obvious by gentle stretching of the skin (Fig. 2).

Her haemoglobin was 11·7 g/dl, the white cell count was normal and erythrocyte sedimentation rate 32 mm/h. The monospot test and the syphilis serology were negative. X-rays of the chest and hands were normal. There was no evidence of bony enlargement or periosteal reaction on radiology.

Histology of a lymph node showed a caseating granuloma, with a small number of acid-fast bacilli seen on direct examination. Biopsy of the soft-tissue swelling of the fingers showed non-caseating granulomata in the deeper dermis. Histology of the lichenoid eruption on her abdomen revealed a granuloma high up in the papillary dermis, typical of the appearances of lichen scrofulosorum (Fig. 3). Her Mantoux reaction was strongly positive at 1:10,000. She was treated with rifampicin, isoniazid and ethambutol. Within a few days her fingers had improved markedly and the fusiform swelling was gone in 2 weeks. The rash on the trunk and limbs cleared within 6 weeks of the start of treatment.

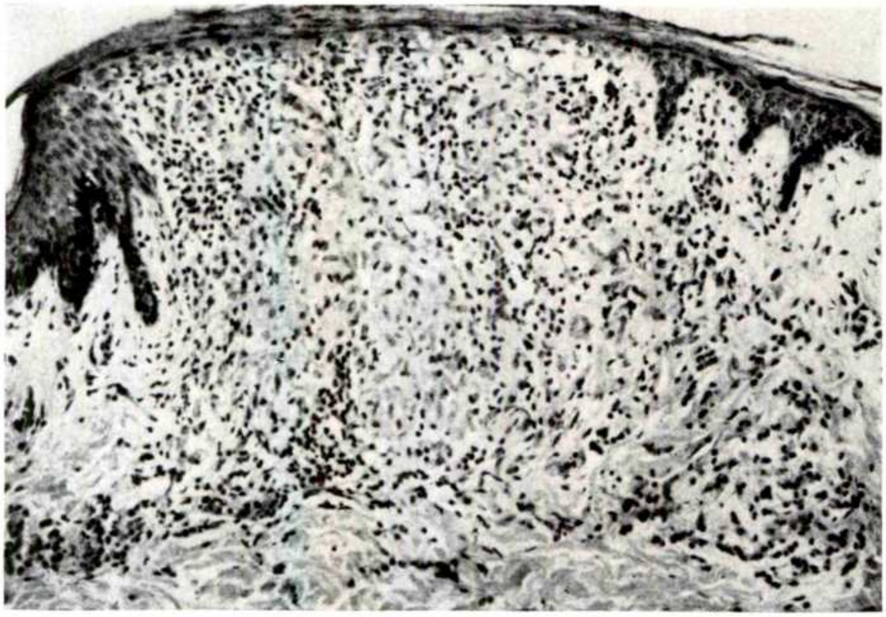

FIGURE 3. Histology of one of the lichenoid papules from the abdomen. There is a non-caseating granuloma high in the papillary dermis, typically seen in lichen scrofulosorum.

COMMENT

Lichen scrofulosorum is one of the tuberculides. Even in the days when tuberculosis was much commoner than it is today, a large series reported by Rauschkolb (1934) contained only eleven examples of lichen scrofulosorum among 6399 cases of tuberculosis. However, the seven cases, including our own, reported in this country in the last 4 years suggest that this form of cutaneous tuberculosis is increasing. The eruption is associated with bony or lymph node tuberculosis and all patients have strongly positive Mantoux reactions suggesting a high degree of immunity (Rauschkolb, 1934). Anti-tuberculous treatment results in complete resolution in a matter of weeks (Smith *et al.*, 1976; Hudson, 1976).

Histologically, the features of lichen scrofulosorum are well-established (Montgomery, 1937). The lesions show non-caseating granulomata high in the dermis, often around hair follicles or sweat ducts but sometimes, as in our case, independent of either.

In addition to the rash, our patient had dactylitis of several fingers. Tuberculous dactylitis is a well-documented entity said to affect single or multiple digits and claimed to be due to underlying involvement of the bone by active infection (Pearlman & Warren, 1961). The affected finger is also said to have unmistakable radiological changes accompanying the swelling. These are said to be characterized by an initial periosteal reaction and, later, progressive expansion and fragmentation of the phalanx or phalanges producing gross transverse enlargement of the bone and overlying fusiform swelling (Hardy, 1958). Although these bony lesions respond to treatment with anti-tuberculous drugs, they take many months to resolve. In contrast, our patient showed no evidence of underlying bony disease, the dactylitis affected a number of fingers in a symmetrical fashion and the swelling subsided dramatically after 2 week's treatment with anti-tuberculous drugs. Histology of the overlying skin showed soft tissue inflammation and granuloma formation. This suggests that there are two distinct types of tuberculous dactylitis, i.e. a condition caused by underlying tuberculous bony involvement and a second, clinically indistinguishable type, due to granulomatous involvement of the skin of the fingers, as seen in our patient.

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Society Proceedings

Proceedings of the 218th Meeting of The Netherlands Society for Dermatology and Venereology, Amsterdam, 23 February 1980

PRESIDENT: DR M.J.WOERDEMAN
HOST: PROFESSOR R. H. CORMANE

SUMMARIES OF PAPERS

Pityrosporum folliculitis: is it a real entity?

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In spite of the fact that pityrosporum organisms can be demonstrated in normal skin and healthy follicles, the mycelium phase of these organisms has been implicated, with little, if any, doubt in the pathogenesis of pityriasis versicolor. These yeasts can probably induce a folliculitis. Occlusion of the follicular ostium by scale formation induced by these yeasts and accumulation of sebum and free fatty acids, by virtue of the ability of these organisms to catabolize fats into fatty acids, would underly the follicular inflammation.

The following arguments support the concept that pityrosporum folliculitis is a real entity, i.e. presence of pityrosporum organism in the inflamed follicle, dermatitis and follicular disturbances can be induced in animals experimentally, and the eruptions respond well to antimycotics.

In nine patients suffering for a long time with itching eruptions on the trunk and extremities, pityrosporum folliculitis was diagnosed. Histopathological findings confirmed the diagnosis.

Pityrosporum folliculitis resembles and has to be distinguished from acne vulgaris in some aspects. The eruptions consist of follicular erythematous papules and little pustules. There are no comedones. Itch is characteristic and sometimes intense. The eruptions are localized mainly on the trunk. Generally patients with pityrosporum folliculitis are older than patients suffering from acne vulgaris.

Predisposing factors in the genesis of pityrosporum folliculitis are (a) treatment with corticosteroids, (b) treatment with antibiotics (e.g. tetracycline), (c) diabetes mellitus.

Pityrosporum folliculitis is probably a common illness. Recognition and special treatment will release the patient from an uncomfortable disease.

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Dermatitis as a side effect of long term treatment with β-adrenoreceptor blocking agents

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In recent years β-adrenoreceptor blocking agents (β-blocking agents) have been frequently prescribed particularly for cardiovascular diseases. There are many reports concerning side effects of practolol, e.g. sclerosing peritonitis and diseases like lupus erythematosus. Because of these side-effects this drug has not been available since 1975. From recent reports it seems that other β-blocking agents also have adverse reactions after long term use. Dermatitis has been reported in many cases of long term treatment with alprenolol, atenolol, labetalol, metoprolol, oxprenolol, pindolol and propranolol (Faure, Hermier & Perrot, 1979; Jensen *et al.*, 1976; van Joost & Neumann, 1980; Neumann, van Joost & Westerhof, 1979; Neumann & van Joost, 1980). Recently we have seen several patients in our departments with dermatitis as a side effect of long term treatment with various β-blocking agents. Although the different β-blocking agents are not similar in chemical structure, they can often cause the same adverse reaction. During long term treatment with β-blocking agents special attention must be paid to the development of psoriasiform and eczematous skin reactions.

The adverse reactions may be mediated immunologically, i.e. blocking of β-receptor on the T-lymphocyte or breakdown product of the β-blocking agents acting as hapten. But the mechanism could also be a pharmacological one via blocking of the β-receptor of epidermal cells and a lowering of the intracellular cyclic AMP which results in more mitosis and which could be responsible for the psoriasiform eruption.

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Clinical applications of cryosurgery

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Cryosurgery was performed for the treatment of basal cell carcinoma and actinic keratosis, as well as for several non-malignant skin diseases such as cutaneous leishmaniasis, chromoblastomycosis, eccrine poroma, syringoma and angiolymphoid hyperplasia with eosinophilia.

The basal cell carcinomas were treated with double freeze-thaw cycles and the extent of freezing was checked by means of sub-/perilesional temperature measurements.

Twenty-five basal cell carcinomas, treated more than 3 months before, were found to be cured with slight atrophy and generally with hypopigmentation. In one instance, delayed wound healing due to a staphylococcal infection was observed.

The non-malignant skin diseases were treated with less intensive cryosurgery, with a good result.

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Photo(chemo)therapy in pityriasis lichenoides

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Pityriasis lichenoides (PL) is a skin disease of unknown aetiology and pathogenesis, which manifests itself in either an acute or a chronic form. The latter form seems rather resistant to all kinds of therapy used until now. Six patients received photo(chemical) treatment. In each case the diagnosis has been confirmed histopathologically. Direct immunofluorescence was not consistent with the existence of an immune-complex vasculitis (Faber & van Joost, 1980). The following table gives a summary of treatment and results.

Patient	Duration of disease until therapy (months)	Nature of therapy	Duration of treatment (days)	Number of exposures	Total Energy (J/cm^2)	Results	Recurrence (months)	Duration free of recurrence (months)
1 (a)	4	PUVA	37	16	34·0	+	1	
(b)	7	PUVA	52	21	44·1	+	2·5	
2	48	PUVA	113	41	316·5	+		14
3	14	PUVA	67	30	56·0	+		2
4	48	PUVA				−		
5	102	Solarium	89	34	*	+		5
6 (a)	6	PUVA	124	45	344	−		
(b)	23	PUVA	243	42	264	+		18

* = Not measured; + = indicates successful therapy; − = indicates unsuccessful therapy.

From these results the impression was gained that the period free of lesions is longer with this therapy than with those used in the past on such patients, for example antibiotics and cytostatics. Four out of six patients are free of lesions up till now (maximum 18 months). A seasonal influence could not be ruled out.

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Regional intravascular sympathetic (RIS) block with guanethidine

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The peripheral blood circulation is to a great extent controlled by the sympathetic nervous system. A sympathetic block causes peripheral vasodilatation resulting in increased skin blood flow. The effect on muscle blood flow remains controversial. Recognition of the beneficial effect of a sympathetic block on peripheral circulation resulted in appropriate therapeutic developments such as chemical and surgical sympathetic blockades and systemically administered drugs with vasodilator properties. However, the dose of these drugs is limited by systemic toxicity so that maximum vasodilator effects cannot be obtained.

Guanethidine (Ismelin), a sympatholytic agent, has been available for the treatment of hypertension for many years. The clinical use of guanethidine as a vasodilator has been restricted by the possibility of untoward systemic side effects. These side effects can be avoided if guanethidine is administered locally.

A possibility to achieve this is by the method described by Bier (1908) for regional anaesthesia in a limb, by intravenous injection of a local anaesthetic after total occlusion of the blood circulation. Using this technique, Hannington-Kiff (1974) produced an effective and prolonged sympathetic block using guanethidine. When injected into a vein guanethidine can reach, probably by diffusion, the neurovascular bundles. It has a selective action on the sympathetic nervous system and is taken up and stored in the post-ganglionic adrenergic nerve endings where the drug depletes noradrenaline stores and blocks the release of catecholamines normally produced by nerve stimulation. The rapid 'fixation' in the tissues and the long half-life of the drug are responsible for a prolonged pharmacological effect.

Possible applications of the RIS block method are:

(1) Diagnostic—to examine if too high an activity of the sympathetic system plays a role in a disease and if vasodilatation has a beneficial effect.
(2) Prognostic—to gain insight into the clinical benefit of an eventual sympathectomy.
(3) Prophylactic—in situations where there is threatening ulceration, gangrene and skin necrosis.
(4) Therapeutic—as supplementary therapy in a number of circulatory and neurological disorders, and to create a more favourable condition for wound healing.

In our dermatological ward, studies are underway on the effect of RIS block on the healing of chronic leg ulcers with an arterial as well as a venous aetiology.

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Chlamydia trachomatis: a retrospective analysis of patients with a positive urogenital culture test

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There is only scant information about the incidence of infection with *Chlamydia trachomatis* (CT) in the Netherlands. The pathogenic significance of CT in sexually transmitted diseases is generally accepted though not definitely proven. In advance of several prospective investigations which are to be undertaken in our clinic, a retrospective analysis was done over a period of 18 months among 159 patients (119 males and forty females) in whom CT could be isolated from the urogenital tract. Of the 119 male patients only four did not have any complaints, whereas fifteen of the forty females were free of symptoms. The spectrum of the complaints was very divergent.

In men there seemed to be a difference between CT and gonococcal urethritis with respect to the length of complaints (mean 36·6 days) and the characteristics and time of the discharge (mucoserous more than purulent and usually in the morning). Homosexuals were notably poorly represented (only four patients). In the past 62·5% of the males, against only 5·7% of the females, had been treated for a venereal disease. Of the tetracycline treated patients (250 mg four times daily for 10 days), 114 could be re-examined for the presence of CT. In four cases the culture test was still positive. Of the 110 patients with a negative control culture test ninety-eight did have symptoms on their first visit. After therapy in seventy-eight of them the symptoms had disappeared; in ten they were persisting and in ten they were not recorded.

The disappearance of symptoms and the negative culture tests following therapy speak in favour of a pathogenic role of *Chlamydia trachomatis*.

In view of the known high transmission rates (up to 70%) and the potential complications of CT-infection (for instance epididymitis, salpingitis, neonatal infections), the tracing and treatment of sexual partners of CT-patients is indicated.

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Psychogenic anogenital pruritus

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Psychogenic anogenital pruritus is a psychodermatosis, in which the absence of dermatological and internal disorders is combined with a typical psychological structure of the patient's character. This structure, for the first time described by Wittkower & Russell (1953), has, in the author's opinion, three typical defence mechanisms (Musaph, 1976):

(1) Reaction formation, e.g. too submissive behaviour in contact with authorities.
(2) Projection, expressed in suspicion and distrust.
(3) Turning the sadistic impulses against the self.

Psychotherapeutic strategy is determined not by the distressing syndrome, but by the personality structure of the patient. We have to find out which part of this structure is our ally in solving the infantile, mostly unconscious, conflict situation which causes the syndrome.

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An ethological approach to scratching behaviour

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The present study illustrates how formal behaviour recordings and analysis, according to ethological standards, can lead to a characterization of a type of situation which involves a high risk of self lesioning. It also permits a detailed and systematic study of the efficacy of various therapeutic procedures in the individual patient.

An eight year old girl, suffering from infantile eczema, was observed during 10 days and 2 nights, by video recording. The behaviour data were collected by use of a manually operated event recorder, which consists of a keyboard, electronic clock and a papertape puncher. Pressing the key, or releasing it, resulted in punching of the time and the code of that key. The record concerned mainly various forms of self-stimulating behaviour, namely scratching, rubbing and leg-brushing.

If one compares the night observation to the day time observations, it is shown that the destructive potential of self stimulation was substantially greater during the night. Further analysis of the behavioural time structure during sleep taught us that there were periods of 'active' sleep during which there was a high risk of self-lesioning.

Finally, the probability is described per time unit of transition from one type of activity (A) to another type (B). It gives a quantitative representation of the recorded behaviour, including sequence, duration and frequency. These results demonstrate how a typical sequence of activities can provoke or inhibit scratching behaviour.

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CASE REPORTS

Congenital ichthyosiform erythrodermia treated with RO 10-9359 (Tigason)

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J.W., male, aged 19 years.

History. Shortly after birth, the patient's skin showed a dull erythema and moderate scaling. The condition rapidly deteriorated over a few weeks. He was treated several times in the clinic by UVA irradiation, with local application of different ointments, and with vitamin A-acid (al-trans form, orally as well as topically). The results of these treatments have always been moderately successful. The patient is in good health. Similar skin disease was not present in any of his relatives.

Examination. A generalized eruption consisting of scaly hyperkeratosis was seen, most intense in the flexures (Fig. 1). Thickened horny plaques and warty nodules were also present on the face and scalp. The skin around the mouth, the anus, the pubic area and the nails seemed to be completely normal. Hair growth was sparse. Rigidity of the periorbital skin produced moderate ectropion. Corneal abnormalities were absent.

Histopathology. All layers of the epidermis were thickened. The papillary ridges were elongated and irregular. Sweat and sebaceous glands appeared normal. The dermis showed some perivascular infiltrate, predominantly lymphocytic.

Treatment. The patient was treated orally by 1 mg per kg-bodyweight RO 10-9359 (Tigason) and topically by glycerine. This treatment produced a rapid and definite improvement and within a week only moderate generalized erythema with pityriasiform scaling could be seen (Fig. 2). After 2 weeks we decreased the daily dose to 0·5 mg Tigason per kg-bodyweight, which was sufficient to maintain the good results. Further diminution of the dose was not possible because of reappearance of the skin abnormalities.

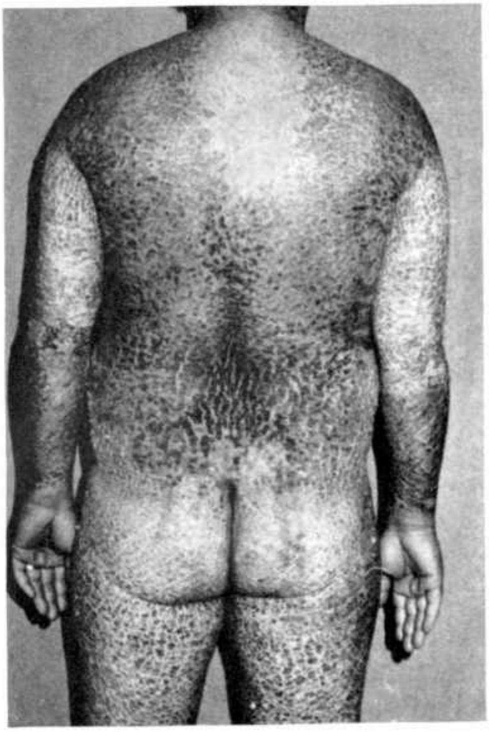

FIG. 1.

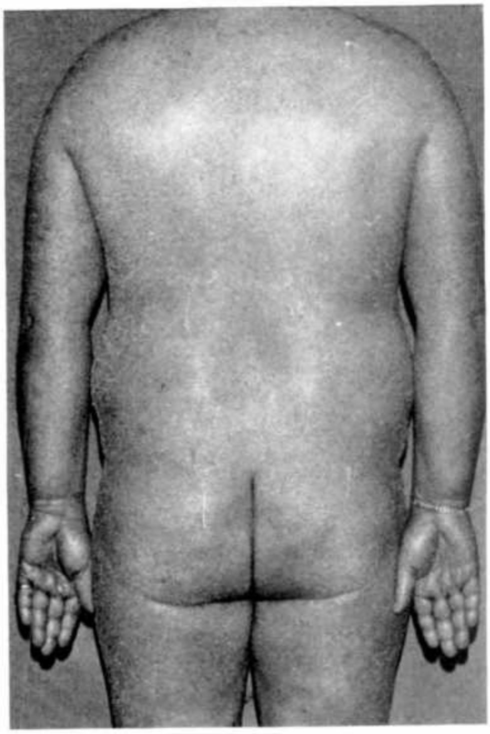

FIG. 2.

FIGURE 1. Congenital icthyosiform erythrodermia before treatment with RO 10-9359 (Tigason).

FIGURE 2. Same patient as in Fig. 1, during treatment.

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MED-testing in a patient with porphyria variegata and vitiligo

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To assess the role of epidermal melanin as a light filter, minimum erythema doses (MEDs) were determined in a patient with porphyria variegata associated with vitiligo. Vitiliginous as well as normal pigmented skin was irradiated with monochromatic light of 310, 405 and 500 nm. The energy required to elicit threshold erythema in vitiliginous skin at 310 nm was two times lower than that for pigmented skin.

At 405 nm the difference in MED estimated in non-pigmented and pigmented skin was four-fold and at 500 nm two-fold.

If the erythema after UV-B irradiation is generated in the dermis, a protection factor of about eight would have been expected.

From these data it can be concluded that UV-B has its erythematogenic action almost exclusively in the epidermis and that the porphyrin phototoxicity in porphyria variegata induced by visible light (405 nm and 500–600 nm) takes place in the dermis.

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Lichen sclerosus et atrophicus around urostoma

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J.B., female, aged 70 years.

History. In May 1978 an urethero-ileo-cutaneostomy was performed according to Bricker, because of urinary incontinence after a vulvectomy with partial removal of the urethra due to squamous cell carcinoma. There was no evidence of pre-existing lichen sclerosus.

In May 1979 she was seen with skin changes around the urostoma that gave problems with the attachment of the bag (using Stomahesive).

Examination. Around the urostoma the skin was indurated (Fig. 3). The epidermis appeared atrophic with bulla formation. The skin colour was lighter than the surrounding skin, the vascular pattern was pronounced and there were small patches of haemorrhage. In the surrounding skin there were small follicular papules.

Differential diagnosis. Sclerodermiform reaction with bulla formation, bullous pyoderma, pemphigoid, mechanical bulla formation, contact allergy.

Investigation. Histology showed pronounced subepidermal oedema leading to bulla formation (Fig. 4). The epidermis was partially atrophic with some follicular plugging. Locally in the basal layers there was a pronounced exocytosis of lymphoid cells and there was a vacuolar degeneration of basal cells. The oedematous papillary layer showed extravasated erythrocytes. Elsewhere in the dermis, reaching as far as the subcutis, there was a pronounced inflammatory infiltrate, with a tendency to perivascular localization, consisting of lymphocytes, histiocytes, plasma cells and eosinophils. Histology was in agreement with lichen sclerosus et atrophicus with septal panniculitis. Bacteriological cultures were negative. Immunofluorescence studies for the presence of immunoglubulins and complement in skin gave negative results by direct as well as by indirect techniques.

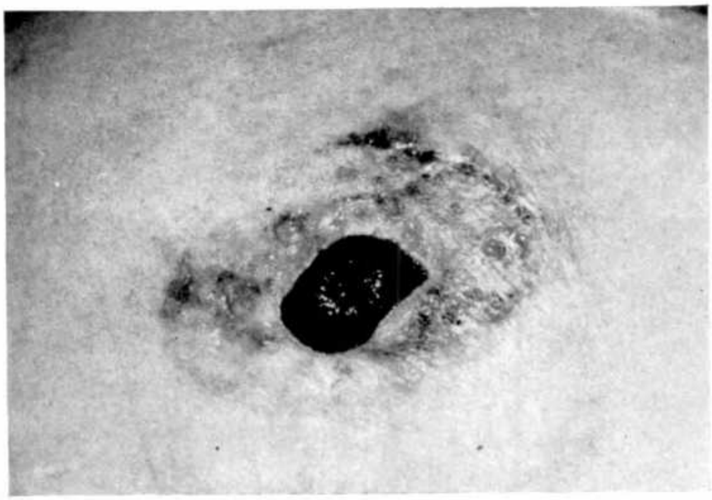

FIGURE 3. Lichen sclerosus et atrophicus around urostoma.

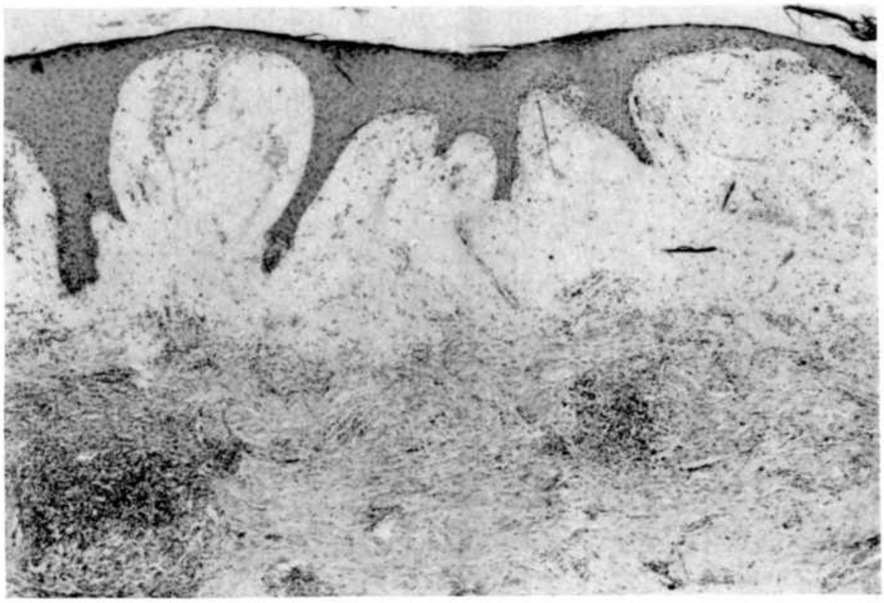

FIGURE 4. Lichen sclerosus et atrophicus.

Bowel-bypass syndrome

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L.K., female, aged 34 years.

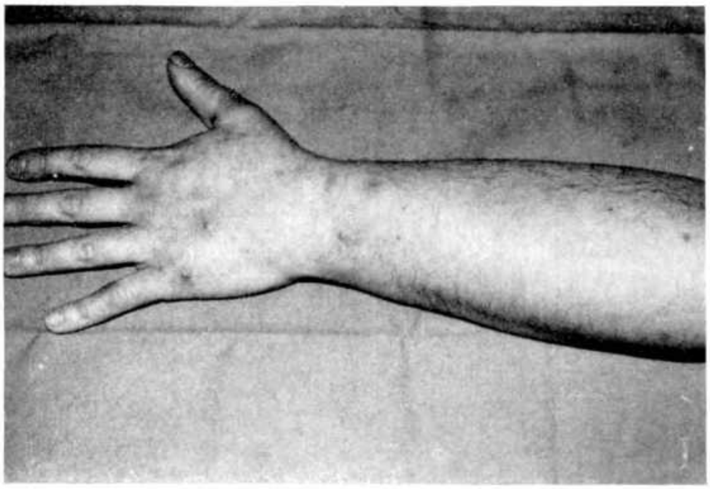

FIGURE 5. Bowel-bypass syndrome, pustular lesions on the forearm.

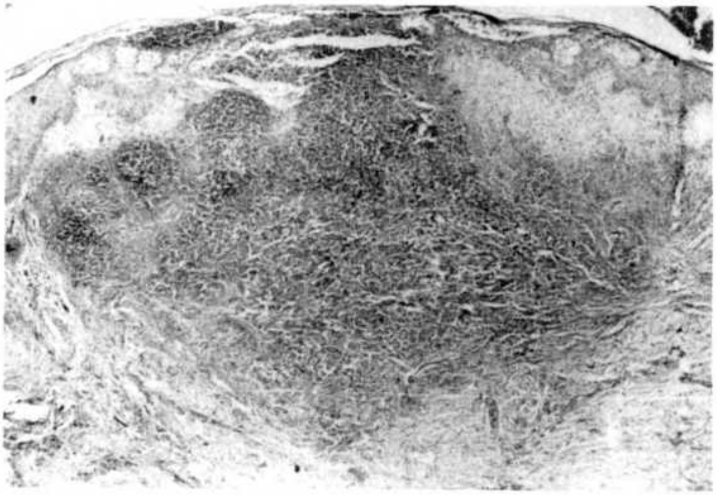

FIGURE 6. Bowel-bypass syndrome, massive infiltrate consisting mainly of neutrophils.

History. In 1974 and in 1976 an end to side jejuno-ileal bypass operation had been undergone for obesity. Since 1979 she has had migratory joint pains in the small joints of hands and feet, ankle, knee, elbow and shoulder. She also complained of diarrhoea. In April 1979, 3 weeks after a breast reduction operation, she developed small pustular lesions all over the body. There was a correlation between the severity of the skin lesions and of the arthralgia.

Examination of the skin. Pustular lesions surrounded by erythema were seen mainly on the upper extremities (Fig. 5).

General physical examination. Weight 91·4 kg; height 1·59 m; no joint swelling.

Histopathology of the skin lesions. There was necrosis of the epidermis (Fig. 6). The dermis contained a massive infiltrate consisting mainly of neutrophils, but no signs of vasculitis. Immunofluorescence examination showed only C3 deposition along some vessels.

Laboratory results. ESR 38 mm/h; Hb 8·8 mmol/l; WBC 7·2 × 10^9/l; urinalysis, serum creatinine, SGOT, SGPT, alkaline phosphatase, antinuclear antibody, hepatitis B antigen, rheumatoid factor, cryoglobulins, circulating immune complexes, C1q, C3, C4 and HLA-B27 all gave normal values. C5 was slightly elevated, 168 g/l (N = 90–130). A bacterial culture of a skin lesion contained no pathogens.

Treatment. With tetracycline 4 × 250 mg daily the skin lesions and arthralgia disappeared. When the treatment was interrupted both arthralgia and skin lesions relapsed.

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Sarcoidosis?

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R.F., male, aged 18 years.

History. The patient was born in Surinam, but has been living in Holland for 8 years. In June 1978 he came to his physician with swollen legs, and to a lesser extent swollen arms, especially in the evening. At the same time scaly lesions developed on the lower legs. Some weeks later annular lesions started on the thighs and spread to the buttocks, hips and forearms. Furthermore he had vague complaints of pain in his left side under the ribs. In the past he had not had any tropical disease as far as was known.

Examination. On the front of the lower legs there were adherent scaly lesions with a craquelé appearance. Dermal infiltration was not present. Both ankles showed mild pitting oedema. On the forearms (Fig. 7), thighs, hips and buttocks there were annular or arciform, slightly elevated lesions without atrophy. Some nerves were thickened but cutaneous sensation was normal.

General physical examination. The liver was enlarged to two fingers below the costal margin with a sharp border. The spleen was massively enlarged as far as the iliac crest. There were no pathological lymph nodes palpable. The eyes were normal.

Laboratory results. Urine: albumin +, glucose −, sediment—a lot of granular casts. Faeces examination negative for occult blood and parasites. ESR 8 mm/h, Hb 7·5 mmol/l, leucocytes 4·1 × 10^9/l with a normal differential. The red blood cells were hypochromatic with anisocytosis, poikilocytosis, Howell-Jolly bodies and ovalocytes as found with hypersplenism. No sickle cells were found. Creatinine 104–120 μmol/l (slightly elevated); Ca 2·47 mmol/l; PO_4 1·02 mmol/l (normal). SGOT/SGPT 25/16 U/l (normal). Alk.phosph. 140 U/l. γ GT 70 U/l (elevated). LDH 289 U/l, haptoglobin 14 μmol/l (as found with haemolysis), bilirubin normal. ANF negative. Au-antigen and antibody negative. Syphilis serology negative. No bilharzia or filaria antibodies have been shown. Brucella titre 20. Monospot negative.

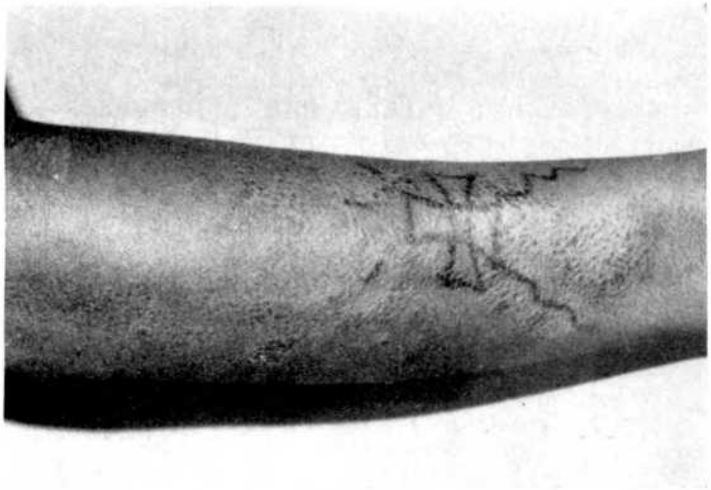

FIGURE 7. Sarcoidosis: annular, partly arciform lesions on the forearm.

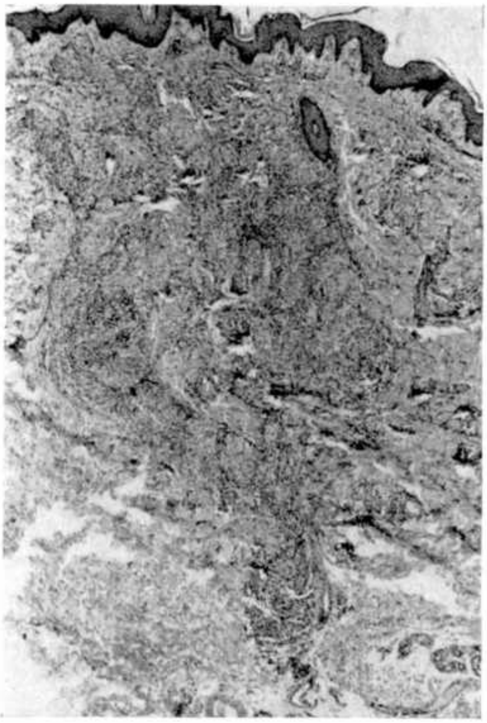

FIGURE 8. Histopathology consistent with sarcoidosis.

Sabin-Feldman negative. Cytomegalovirus titre negative. Mantoux negative. Lepromin test negative after 72 h, and 3 mm after 4 weeks. Kveim test negative. Angiotensin converting enzyme 144 ($N < 52$). Lysozyme 10 mg ($N < 2$). No malaria parasites or microfilaria have been seen. Bone marrow showed active haematopoiesis. Chest-X-ray normal. Gallium scintigraphy of the thorax: increase of activity in the lower lung fields. Scan of liver and spleen showed an homogenous enlarged liver and an enormous enlarged spleen, with slightly irregular activity; no clear signs of cirrhosis.

Histopathology. In the pigmented skin the changes were mainly in the dermis (Fig. 8). They consisted of small granulomatous infiltrates of histiocytes and foreign body giant cells with a small number of lymphocytes around. The granulomas, which showed no central necrosis, were arranged in irregular patterns. In biopsies of the liver and the spleen the same granulomatous lesions were seen. No fungus or acid-fast bacilli were found.

Conclusion. By excluding other diseases with granulomatous infiltrations the diagnosis of sarcoidosis seems the most appropriate, notwithstanding the unusual presentation.

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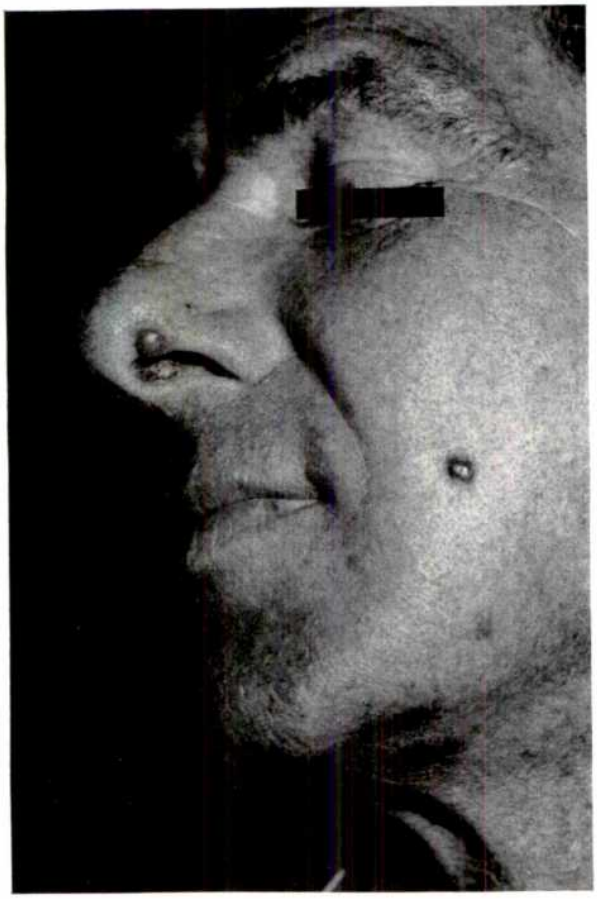

FIGURE 9. Angiolymphoid hyperplasia with eosinophilia; dome-shaped tumours on the face.

Angiolymphoid hyperplasia with eosinophilia

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J.A., male, aged 46 years.

History. A tumour was first noted in 1974 on the scalp of a 46-year-old man who l from psoriasis for 30 years. This was excised at another hospital (histopathology u months a new tumour developed at a different site on the scalp. Afterwards more tu They were not painful and never bled. The patient used topical treatment for his ps these tumours.

Skin examination. He had psoriasis on the trunk and limbs. On the left side of the fa fifteen dome-shaped tumours were seen ranging in diameter from several mm to ab They had a livid red colour, a firm consistency and were not fixed to the underlying t side of the neck some subcutaneous nodules were palpable.

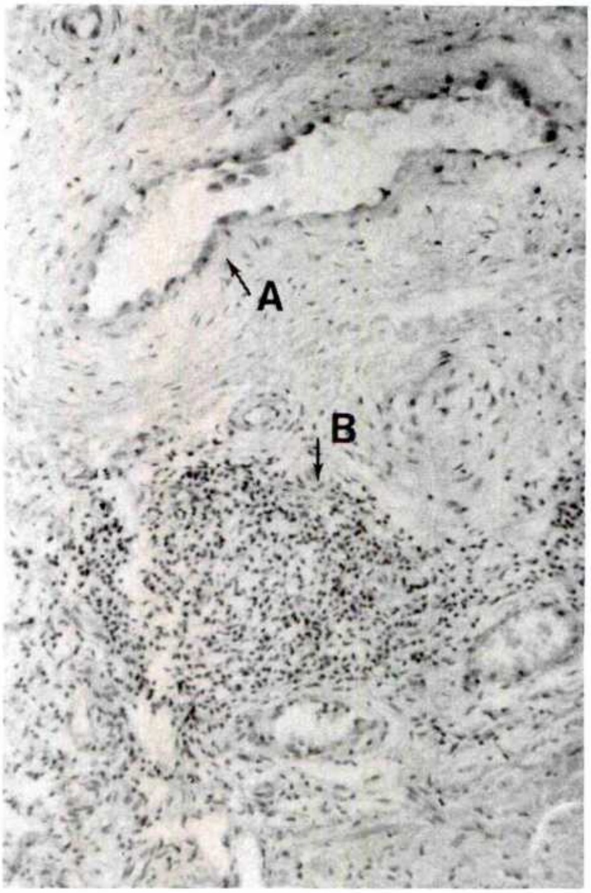

FIGURE 10. Angiolymphoid hyperplasia with eosinophilia; A—vessel wall, B—ir

General examination. Revealed no internal diseases.

Laboratory tests. Hb 9·8 mmol/l, ESR 7 mm/h, leukocytes normal (no eosinophilia). Liver and kidney function tests normal. Blood sugar normal. ECG normal. X-rays of chest and skull: no abnormalities. Left external carotid arteriogram: no abnormalities. Test for cellular immunity: normal delayed reactions for several viral, bacterial and fungal antigens.

Histopathology. Examination of several biopsies revealed numerous small vessels with plump, atypical endothelial cells projecting into the vascular lumen (Fig. 10). The nuclei of the endothelial cells are variable in size and in several instances vacuolization is seen. The vascular walls are not typical of either arteries or veins. Scattered through the dermis are mononuclear cells and eosinophilic granulocytes. Within the areas of vascular proliferation are sporadic foci of lymphocytes.

Immunofluorescence examination. There is sporadic C3 deposition in the small vessels. No immunoglobulins detectable.

Therapy. Firstly many of the tumours were treated by excision by the plastic surgeon. Afterwards, cryotherapy for superficial lesions was successful.

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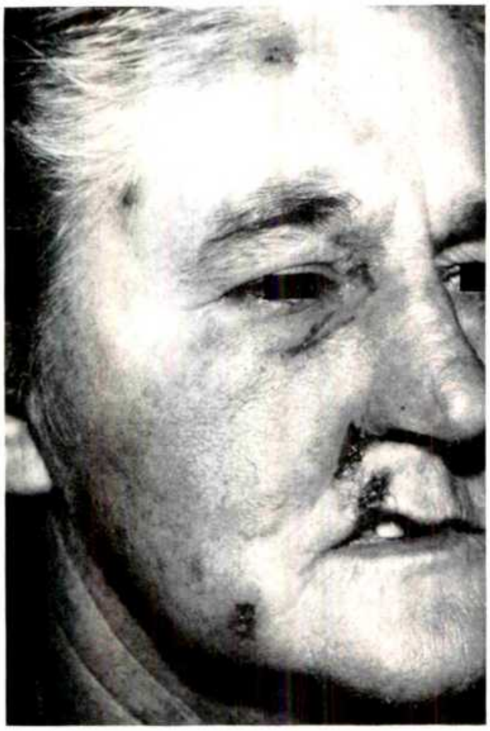

FIGURE 11. Neurotrophic ulcers of the face after section of the trigeminal nerve root.

Neurotrophic ulcers of the face (trigeminal trophic syndrome)

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T.N.C., female, aged 56 years.

History. After 10 years of severe trigeminal neuralgia, not responding to drug therapy, a 56-year-old woman was operated on in 1978 (section of the trigeminal nerve root according to Dandy). Two months after this operation small ulcers appeared, initially at the right side of her nose, later elsewhere on the involved side of the face. She complained of paraesthesiae in the right nasal mucosa, and did not deny picking at the involved areas.

Examination. Multiple superficial, sharply delineated ulcers on the right half of the face (Fig. 11). Sensation to pressure and temperature absent. No evidence of exposure keratitis. Corneal reflex negative.

Laboratory tests. ESR 26 mm/h, no other abnormalities. Culture from ulcer: penicillin resistant *Staphylococcus aureus*, no *Candida albicans*.

Thermography face. The right half of the face is cooler than the (normal) left, except at sites of ulceration where the temperature is raised. Sweat test: anhidrosis of the right half of the face.

Discussion. The aetiology of this syndrome is unknown, but a disturbed picking inhibition reflex, trauma, and distorted micro-circulation as a result of autonomic denervation are probably important factors.

Treatment. No specific treatment is available; systemic antibiotics were without success. Some improvement occurs after covering the ulcers with non-adherent bandages (Metalline).

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Comment

REPORT ON A MEETING ON THE PROPERTIES OF MELANINS

In recent years there have been a number of studies on the unusual biophysical properties of natural and synthetic melanins. At an international meeting devoted to the 'Biophysics and Biological Functions of Melanins' in Parma (Italy), on the third and fourth of March 1980, an attempt was made to integrate many aspects of these characteristics with the chemical and biological knowledge about these pigments. The full proceedings will not be published. The following is a brief account of the meeting.

Some of the difficulties in the chemical analysis of synthetic melanins were detailed by H.Wyler (Lausanne), notably the 30% loss of labelled β-carbons during formation of dopa melanin. Nevertheless, the combined data from elemental analyses and oxidation equivalent titrations show a remarkable correspondence between dopa melanin and melanin from sepia ink, and the stochiometry is consistent with the conventional linear indolic polymer model for animal eumelanin, although doubts remain about the actual structure.

Evidence for the existence of three different hydration states of melanin in melanin granules was discussed by P.R.Crippa (Parma), and bulk water (A band) hydration to the extent of 15 water molecules per polymer estimated.

All natural melanins, especially those from vertebrates, contain significant amounts of sulphur. G.Prota (Naples) described the chemistry of sulphur incorporation in terms of nucleophilic addition to dopaquinone of sulphydryl compounds, such as cysteine and glutathione, and the subsequent formation of cystein-S-yldopa intermediates which are incorporated into the polymer as confirmed by degradation studies of some purified pigments from mammalian eyes. In line with this proposed mechanism, H.Rorsman (Lund) reported evidence that significant amounts of cystein-S-yldopas are indeed produced *in vivo* in all melanogenic tissues and are partly excreted in the urine. The possibility of using this as an indicator of melanogenic activity in malignant melanoma was rendered difficult by the pronounced variation in urinary cystein-S-yldopa levels with exposure to sunlight. R.C.Sealy (Wisconsin) presented evidence that at least part of the photoprotective action of melanins can be accounted for in terms of reversible internal free radical reactions involving an equilibrium between semi-quinones and a mixture of quinones and hydroquinones. This would furnish an explanation both for the 'stable' free radical nature of the polymer and a mechanism for the conversion of absorbed photon energies into heat. In addition, intramolecular electron exchanges of this kind enable melanins to exhibit remarkable electron transfer properties, as described by I.A.Menon (Toronto) for ferricyanide reduction linked to NADPH oxidation with melanin as the intermediary in the electron transport chain. Electron transfer in this system was facilitated by illumination.

The value of some specific properties of the electron spin resonance (e.s.r.) signal of melanins in clinical diagnosis was discussed by H.M.Swartz (Illinois), particularly in relation to the insensitivity of the melanin e.s.r. signal to boiling and its special interaction characteristics with transitional metals. The properties of copper (II)-binding by melanin detected by e.s.r. spectroscopy were reported by T.Sarna (Krakow) who proposed four types of metal ion binding by melanins of which two, catechol and combined carboxyl and amino group interaction, were significant in the physiological pH range. The ion-exchange properties of melanins were also investigated by B.Larsson (Uppsala), who reported on the characteristics of the uptake of metals and a range of melanophilic drugs. With the exception of chlorpromazine the binding was consistent with a mechanism based on ionic binding.

D.Slawinska (Poznan) reported evidence of singlet oxygen formation by a wide range of photo-

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oxidized melanins as well as radical scavenger properties of non-illuminated melanin. Evidence that epidermal melanin is less photo-protective in the higher energy region where the absorption is maximal, based on minimum erythema doses on normal and vitiliginous skin of a patient with porphyria variegata, was presented by W.Westerhof (Amsterdam). N.VanTieghen (Brussels) showed that exposure of HM6B melanoma cells to dihydroxyphenylalanine reduces the proportion of cells exhibiting tyrosinase activity.

Evidence that selective cytotoxic damage to melanocytes may be caused by free radical intermediates formed during the oxidation of tyrosinase substrates was presented by P.A.Riley (London).

J.E.McGinness (Houston) showed that melanins possess unusual semiconductor properties and are capable of metastable switching behaviour when an electric current is passed through the polymer. Remarkable temperature rises induced by phonon absorption by melanins also occur. R. Kono (Tokyo) reported on the excited electronic states produced in synthetic melanin by ultrasound. The process, which is coupled to alterations in the semiconductor properties of the pigment, is analogous to the effects of photon absorption and the generation of free radical species may contribute to the cytotoxicity to melanized tissue of ultrasound irradiation. On the other hand, there is tissue of ultrasound irradiation. On the other hand, there is evidence (reviewed by L.Lyttkens (Uppsala) that pigmentation of the inner ear has a protective effect against noise, although the mechanism is unknown. P.H. Proctor (Galveston), surveyed evidence that, on the basis of its electronic properties, one of the functions of melanin in non-illuminated areas might be to act as a free radical or excited-state energy sink, particularly in relation to modulating tissue levels of superoxide.

In discussion, it became evident that there were difficulties in comparability of the results from different laboratories because of the lack of standardization in the preparative procedures and the description of material. Doubts were expressed about the usefulness of the present nomenclature in which melanins are classified as eumelanins, phaeomelanins and trichochromes. It was agreed that, in the absence of a rigorously defined structure, an operational definition of melanin should be adopted in which standardized methods of preparation are used, yielding material of known molecular weight, nitrogen and sulphur content, and degree of hydration.

G.PROTA
P.A.RILEY

British Journal of Dermatology (1980) **103**, 583.

Correspondence

RAZOXANE IN THE TREATMENT OF PSORIASIS

SIR, We should like to comment on the article by Dr Atherton and colleagues (*British Journal of Dermatology*, 1980, **102**, 307) on the treatment of psoriasis with razoxane and their conclusions regarding its superiority over methotrexate.

Whilst hepatotoxicity, cirrhosis and fibrosis in particular, is a well documented phenomenon following long term treatment with methotrexate, published evidence shows that these effects are uncommon (Zachariae, Grunnet & Sogaard, 1975) and that the pathogenesis is multifactorial, depending on such factors as alcoholic intake, age, obesity and pre-treatment liver histology in addition to cumulative dose and schedule of methotrexate therapy (Nyfors & Poulsen, 1976). By undertaking liver biopsies before and during treatment and utilizing an intermittent dosage schedule (Warin *et al.*, 1975), the incidence of hepatotoxicity can be kept to an acceptable level.

The conclusion of Dr Atherton *et al.* that razoxane does not cause hepatotoxicity must be considered premature, based as it is on liver function tests, which incidentally have been shown to correlate poorly with histological findings (Reese *et al.*, 1974), and two liver biopsies.

The unsubstantiated assertion that methotrexate is carcinogenic is incorrect. Unlike most cytotoxic agents, methotrexate does not react chemically with DNA nor is it incorporated into nucleic acids, its action being wholly attributable to inhibition of the synthesis of essential DNA components (Jackson & Niethammer, 1975). Contrary to Dr Atherton and colleagues' unreferenced statement, methotrexate has in fact been shown to be negative on the Ames test (Benedict *et al.*, 1977) and an increasing body of clinical evidence supports the contention that methotrexate is not carcinogenic in humans (Bailin *et al.*, 1975; Bagshawe, 1977). These clinical observations are further supported by the results of long term toxicity studies in rats which show that methotrexate does not induce chromosomal aberrations and is not carcinogenic (Unpublished data, Lederle Laboratories, 1973).

The therapeutic benefit to be gained from the use of razoxane in the treatment of psoriasis is not in dispute, but it would appear to be somewhat early to draw conclusions about any therapeutic superiority over methotrexate or any advantage in terms of safety and tolerability.

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Reply

SIR, Dr G.W.R.Hill and Miss M.Roach are concerned that premature conclusions may be drawn from our recent paper (Atherton *et al.*, 1980) with regard to the role of razoxane vis-à-vis methotrexate in the treatment of severe psoriasis. It was not our intention to imply a general recommendation that razoxane be preferred to methotrexate whenever a systemic antimitotic drug is indicated in such patients. Indeed, we were cautious in avoiding such a implication. While we entirely agree with Dr Hill and Miss Roach that no overall conclusions can be drawn at this time as to the relative efficacy of these two agents, there can be little question that there is a clear role for razoxane where methotrexate has not proved adequately effective, or where hepatotoxicity has led to withdrawal of methotrexate therapy. Our treatment group included both categories of patient. These patients had been referred from other centres, and the conclusions of their previous doctors with respect to their previous methotrexate treatment were naturally accepted without question.

In our view, the hepatotoxicity associated with methotrexate *does* constitute a real problem in the treatment of psoriatic patients. Zachariae, Kragballe & Sogaard (1980) have recently published the results of serial liver biopsies in large numbers of methotrexate-treated psoriatics, and their report is the most comprehensive survey to date of liver disease in relation to methotrexate therapy. Their finding, that cirrhosis developed in a quarter of patients treated for more than 5 years, hardly justifies Dr Hill's and Miss Roach's claim that methotrexate hepatotoxicity 'is not generally a clinical problem'. Our suggestion, that razoxane is less likely to be hepatotoxic, is based not on our own very limited data, but upon the complete absence of any report of such an effect in several thousand patients so far treated with razoxane for malignant disease. For this reason, we do not consider that routine liver biopsies are justified in patients receiving razoxane. Clearly it is nevertheless not possible at this stage to rule out completely any risk to the liver from razoxane.

Dr Hill and Miss Roach are justifiably critical of our implication that methotrexate is mutagenic in the Ames test. As they say, the test has been negative for methotrexate. Methotrexate has however been shown to induce marked chromosomal aberrations (Ryan, Boddington & Spriggs, 1965; Krogh-Jensen & Nyfors, 1979), which, it is assumed, reflect damage to DNA. Similar chromosomal damage is observed in patients with Bloom's syndrome, ataxia telangiectasia and Fanconi's anaemia (Polani, 1979), conditions which are now known to be associated with a high risk of development of malignant tumours. In the light of these findings we believe that a carcinogenic effect of methotrexate must be assumed to be a distinct possibility until proven otherwise. While it is undoubtedly true that a statistically significant increase in incidence of malignant disease has not been demonstrated in psoriatics treated with methotrexate (Bailin *et al.*, 1975), the control population was not a psoriatic one, and it has been suggested that the incidence of malignant disease may be generally reduced amongst psoriatics (Shuster, Chapman & Rawlins, 1979). Furthermore, the report of development of hepatoma in a child with methotrexate-associated cirrhosis (Ruymann, Mosijczuk & Sayers, 1977) points to a definte risk in relation to this specific type of malignancy. The undoubted teratogenicity of methotrexate (Powell & Ekert, 1971) does not *per se* prove mutagenicity, but does constitute additional indirect evidence for such an effect.

The hazard of neutropenia with razoxane is very real, though it can be minimized by careful follow-up of patients under treatment. For this reason, it is our view that the use of this agent should for the present be restricted to centres around the country where some 'feeling' for the drug can be gained by the experience of treating several patients. It should never be used unless facilities are available for seeing the results of blood counts on the day the patient attends the clinic. Methotrexate has a high margin of safety acutely in respect of bone marrow suppression, and many dermatologists would justifiably favour it for this reason. Any real advantage of razoxane would relate to long term safety, though we entirely agree with Dr Hill and Miss Roach that it is as yet very much too early to draw definite conclusions on this matter.

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Rosacea, metronidazole and pregnancy

Sir, The paper from Saihan & Burton (*British Journal of Dermatology*, 1980, **102**, 443) led us to make the following comments: we agree with the remarkable effectiveness of metronidazole in rosacea as was demonstrated in our open study of sixty cases (Guilhou *et al.*, 1979). But our conclusions concerning the safety of the drug in pregnant women and in long term treatment are quite different. As mentioned by Saihan & Burton, metronidazole has been demonstrated to be mutagenic in some bacteria (Voogel, Vander Stell & Jacobs, 1974). Moreover this drug could be carcinogenic in rodents (Rustra & Shubik, 1972). Saihan & Burton state that 'the significance of this, in man, is uncertain'. This seems a sufficient reason for avoiding metronidazole during pregnancy until more data are reported on possible mutagenic and carcinogenic effects.

Moreover if we can regard metronidazole as a safe drug for short term treatment (Beard *et al.*, 1979), the possible adverse effects of long-term therapy are uncertain. For this reason we think that metronidazole is not to be given for more than 3 months in patients suffering from rosacea or perioral dermatitis.

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Reply

Sir, We thank Professors Guilhou and Meynadier for their letter, which raises three important questions.

Is metronidazole teratogenic?
Metronidazole has been used for the treatment of trichomoniasis in pregnancy for the past 20 years. Careful monitoring of the offspring has been carried out, and several large studies have shown no increased incidence of malformation, abortion or still-birth, even when metronidazole was taken in the first trimester (Berget & Weber, 1972). Animal tests have been equally reassuring, and even in a dose of 100 mg/kg daily throughout pregnancy, there was no sign of teratogenicity (Gauter, Jolou & Cosar, 1960). We agree that all drugs should be prescribed with reluctance during pregnancy, but there appears to be no specific risk from metronidazole (Shepard & Fantel, 1977).

Is metronidazole carcinogenic?
Metronidazole in large doses may be carcinogenic in rodents, but such experiments cannot be directly extrapolated to the use of smaller doses in man (Leading Article, 1980). A retrospective study of 2,460 subjects treated with metronidazole between 1969 and 1973 (Friedman, 1980), and a study of 771 women who took the drug during 1960–69 (Beard *et al.*, 1979), have both shown no increase in cancer attributable to metronidazole. The claim quoted by Guilhou and Meynadier, that metronidazole may be mutagenic in patients with Crohn's disease (Mitelman, Hartley-Asp & Ursing, 1979) has now been withdrawn (Mitelman, Strombeck & Ursing, 1980) since the observed changes were more likely to be due to the sulphasalazine which the patients were also taking. Hartley-Asp (1979), after a fruitless search for chromosomal aberrations due to metronidazole, concluded that 'for all practical purposes we can regard metronidazole as a safe drug for short-term treatment'.

Is metronidazole safe for long-term use?
Metronidazole should probably not be given for more than 3 months, but this is because of the risk of sensory peripheral neuropathy mentioned in our paper, rather than any mutagenicity in bacteria. The neuropathy is usually reversible on cessation of treatment, but in some cases the symptoms have been persistent (Collier, 1980).

We therefore stand by our original statement that 'metronidazole is a worth-while drug for the treatment of papulo-pustular rosacea, especially in patients who have not responded to tetracycline, or for pregnant women in whom systemic treatment is essential'.

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British Journal of Dermatology (1980) **103**, 587.

Book Reviews

Systemic Pathology. Second Edition. Volume 6: Skin, Eyes, Ears. Edited by W.ST.C.SYMMERS (1980) London: Churchill Livingstone. Pp. 406. Price £42.

This is the final volume of a series of six that comprise the whole work 'Systemic Pathology'. The sections on The Eyes and The Ears are interesting but beyond my competence to review critically. The section on The Skin occupies almost three-quarters of the volume and is the work of the late H.Haber and the late J.A.Milne with the final form being the work of the Editor W.St.C.Symmers.

In the preface the Editor expresses a wish for thoughtful constructive criticism in reviews of the work but there is little to criticize in the section on The Skin. The classification adopted is based for the most part on the position of the main abnormality in the skin when an obvious aetiology is in doubt. The lay-out is clear with a double column per page and usually the photomicrographs effectively illuminate the script which is singularly free of spelling mistakes, although weal appears as 'wheal' occasionally. The photomicrographs could have been collected together better to relate to the text, eg. in the sections on Psoriasis, Keratoacanthoma and Sweat apparatus tumours.

There is an uneven emphasis throughout the Skin section with prominence given to skin infections. The section is well written and interesting but Dermatitis is discussed in about the same amount as the combined sections on Darier's disease and Solitary Follicular Dyskeratosis. The microscopic changes in Dermatitis that help to differentiate seborrhoeic and atopic types might have been discussed to advantage. In the section on basal cell carcinoma there is no mention of the superficial type in the clinical differential diagnosis of intraepidermal carcinoma and the histological changes of the superficial form are not clearly described to relate to the clinically described 'Erythematoid type' of basal cell carcinoma. The term 'basisquamous' is used frequently in this section and is a term of doubtful value and is little used in British dermatopathological reporting.

The old and almost certainly incorrect notion that naevus cells migrate from the epidermal basal area to the dermis is perpetuated. Halo naevus is mentioned clinically but the histological features which can be important with reference to malignant melanoma are not. There is no mention of prognostic histological pointers in malignant melanoma such as the grading suggested by Clarke.

In the section on 'Immune Disorders of Connective Tissue' the L.E. cell phenomenon is discussed in detail but the tests for antinuclear antibodies are rather dismissed and the anti-DNA antibody test and its usefulness in diagnosing L.E. are not mentioned.

As already noted, the section on Skin Infections and Infestations is detailed and extensive. The mouse foot-pad is mentioned for the growth of the Lepra bacillus but not the more useful Armadillo as an experimental animal.

There is a helpful section on Lymphomas of the skin but in the section on Parapsoriasis, the laudable attempt to try and order the different terminologies used by dermatologists has probably been too detailed and is likely to confuse the pathologist who has little dermatological experience.

Mention must be made of the many helpful footnotes throughout the text which I particularly enjoyed. For the most part these are guides for the unwary through the minefield of dermatological eponyms, eg. Ito's hypomelanosis and Ito's naevus, but the one discussing sclerema and scleroedema does rather make the mind reel!

This volume is not a definitive textbook on Dermatohistopathology and does not attempt to be but there are some surprising omissions, e.g. nothing on erythropoetic protoporphyria or graft-vs-host reaction. This, and some of the other criticisms, may be associated with the lack of any references after 1976 and very few from 1970 even though over 1300 references are quoted. The specialist dermatopathologist will already have more specific and detailed reference books and at the price will probably not feel the need to buy this book. However, in the context of a textbook of general pathology it is a very useful component The overall price will mean it is available mainly in libraries but any pathology department must benefit from having a copy of its own.

R.H.MACDONALD

Handbuch der Haut-und Geschlechtskrankheiten. J. Jadassohn. Ergänzungswerk. Nicht Entzündliche Dermatosen IIIB. Bösartige Geschwülste-Leukämie. Edited by H.A.GOTTRON and G.W.KORTING (1979) Berlin: SpringerVerlag. Pp. XV,780. Figs 160.

The first of the supplementary volumes of J.Jadassohn's great handbook was published in 1959 under the editorship of A.Marchionini. Since then twenty further volumes have been issued and two more are in preparation. The present volume is entitled 'Non-Inflammatory Dermatoses and Malignant Tumours, Leukaemia'. Its original editor, Professor Gottron, was succeeded on his death by Professor Korting. Like its predecessors this volume presents a systematic review of the literature supported by lengthy bibliographies. These bibliographies are of great value, but they differ in the period they cover. For example, the list of references ending Schimpf's chapter on squamous carcinoma concludes in 1971 whereas Lanseckert's bibliography on skin metastases ends in November 1975. The excellent chapter by Anton Musger on leukaemia gives no concluding date for the bibliography but ends in 1976. This book thus provides a very comprehensive survey of the literature from the date of publication of the corresponding volume in the original series up to the early or mid 1970's. It is not intended to be a review of recent advances but a solid and indispensable permanent record of work published during the stated period; very little that was published during that period has escaped the authors' notice although one or two reputable journals appear, surprisingly, to have done so.

This important addition to an invaluable series should find a place in every dermatological library that can afford it.

A.J.ROOK

Clinical Leprosy. V.N. SEHGAL (1979) India: Vikas Publishing House PVT Ltd. Pp. 100. Price 22 rupees.

This 100-page paperback on leprosy printed at the Cambridge Press, Delhi feels like Jopling's 'Handbook of Leprosy' or Bryceson & Pfaltzgraft's 'Leprosy'. Unfortunately, though written by an enthusiastic teacher with a passionate dedication to his subject and great experience, it cannot be placed in the same class of excellence. The Indian style English can hardly be the best medium 'to cater the need of the students', nor can the text be held 'concised and comprehensive, well illustrated with neat photographs' as the preface tells us. Regrettably the illustrations are of uniformly mediocre quality.

Misprints and misspellings abound—even of a standard drug such as clofazimine which appears regularly as clofazamine, and receives no mention as being valuable in the treatment of Type II reaction. Some errors are even more riveting, such as the statement that 'Orthopods are again being incriminated in the transmission of the disease' (p. 13).

This is all a great pity for, with a little consultation and outside help, all the work and experience put into this small volume could have resulted in a textbook of quality.

The price is modest at 22 rupees (18·5 rupees to the pound sterling).

R.R.M.HARMAN

The Porphyrias. Clinics in Haematology, 9: 2. Edited by A.GOLDBERG and M.R.MOORE (1980). London: W.B.Saunders. Pp. 451.

The clinical manifestations in the skin of the porphyrias could be tabulated in half a page but a full and clear understanding of these diseases is important to the dermatologist and to attain this he requires the factual information of a three to four hundred page book. Opening the pages of many of the compact books on this subject suggests two alternatives to the amateur—to confess to intellectual poverty or to dismiss the unfathomable biochemistry as alchemy. It is very refreshing to find that the volume of 'Clinics in Haematology' devoted to the porphyrias has been written with compassion, resulting in an eminently readable book which covers all the important ground (and a little bit more). The clear and coherent text has been written by a galaxy of stars from the clinical 'porphyrin world'.

Excellent chapters on the biochemistry of the porphyrins as well as the clinical chemistry and diagnosis of the porphyrias are included. Of particular interest to the dermatologist are individual contributions on acute hepatic porphyrias, congenital porphyria and porphyria variegata. There is also a comprehensive but relatively concise chapter on the cutaneous porphyrias.

For departments or for individuals with a library shelf for specialist books I would certainly include this one on the porphyrias.

D.M.MacDonald

The Treatment of Mycoses with Imidazole Derivatives. Wolfgang P.E.Rabb (1980) translated by T.C.Telger. Berlin: Springer Verlag. Pp. 173, b/w illustrations. Price DM 28.

This attractively produced little book includes a rather curious mixture of ingredients. There is a detailed account of the author's laboratory work on the imidazoles (mainly econozole), a useful introduction to the mode of action and pharmacology of the imidazoles, a brief personal account of fungal diseases and an enthusiastic review, mainly from the continental European literature, of the usefulness of the imidazoles in therapy. There are in all 358 references—not a comprehensive list in this busy area! The index is poor, an irritation rather than a major drawback in so small a book. This recipe is very similar to the one the author adopted previously in his book 'Natamycin (Pimaricin), its Properties and Possibilities in Medicine' (1972), but it hardly seems likely to satisfy the needs of most readers of this journal, being inadequate as a practical guide to treatment and incomplete as a review of the literature of the imidazoles. Despite Dr Kligman's laudatory preface in which he praises Dr Raab's unbiased approach, this reviewer finds a lack of critical judgement in this book. Imidazoles may rule, but unchecked enthusiasm for them is not 'O.K.'.

S.O.B.Roberts

Skin Troubles—Hamlyn Pocket Health Guides. London: Hamlyn Paperbacks. Pp. 48. Price £0.65.

This little paperback is written by a freelance journalist specializing in medical subjects and is a simple primer on skin care and common skin ailments. It presents a straightforward, well-informed and well balanced account without the usual shortcomings. It can be recommended.

R.H.Champion

The Year Book of Dermatology—1979. Edited by Richard L.Dobson. Chicago: Year Book Medical Publishers Inc. Pp. 367. Black and White illustrations.

The Year Book of Dermatology is like the Norman Conquest and Magna Carta, a 'good thing' (Seller W.C. and Yeatman R.J., 1066 and All That, London, Methuen, 1930). This at least is the view generally held by clinical dermatologists and the 1979 version edited by Dr Richard Dobson of Buffalo is likely to confirm this opinion. The successful formula continues with a review article provided by James E.Rasmussen on the important topic of percutaneous absorption in children (20 pages of text, 89 references), and abstracts of 335 papers (dating from December 1977 to November 1978 inclusive). As usual the specialist dermatological journals provide most of the entries and inevitably English language publications dominate. The indexing is excellent, all contributing authors, even when there are as many as twelve to a single paper, are listed, and if the subject index is rather short on synonyms it is sound and accurate. My favourite section 'New and Unusual Disorders' seems a little sparse this year but perhaps this is an encouraging sign. There is however no shortage of footnotes. The editor has himself generously provided footnotes for almost every paper in every section. They are all punchy and to the point. Some of them will certainly give food for thought: is potassium iodide really the treatment of choice for kerion ringworm? It appears to work well in Buffalo. There is a quiz at the beginning of the book carefully constructed to reveal the would-be reader's ignorance, the answers to the questions being provided within the abstracts of the papers covered. I looked in vain for any question ending 'and does it matter ?' So characteristic of the test papers in '1066 and All That'. History, according to Seller and Yeatman is now at an end. Dermatology we all know is not, hence the Year Book.

S.O.B.ROBERTS

Living with Psoriasis. LESLIE STANKLER and DAVID J.TEMPLE. Available from The Psoriasis Association, 7 Milton St., Northampton NN2 7JG. Price £0.50, to be donated to the Psoriasis Association.

This is a small eight-page pamphlet aimed primarily at patients with psoriasis.

British Journal of Dermatology (1980) **103,** 591.

News and Notices

Investigative Group Meeting. British Association of Dermatologists—23rd and 24th January, 1981—Cardiff

This meeting will take place all day Friday 23rd and Saturday 24th January 1981, at the Welsh National School of Medicine, Cardiff.

Individuals who wish to be considered for the Investigative Group Travelling Fellowship should send a brief *curriculum vitae* and also confirm that they will be the presenters of a paper to be delivered in Cardiff. (Further details of the Fellowship may be obtained from Professor MacKie if required.)

Four workshops will be held on the Friday. (1) Physical properties of skin. (2) Recent developments in acne vulgaris. (3) Malignant melanoma. (4) The mast cell.

An innovation this year will be a half-day symposium, to be held on the Saturday morning, on 'Hormone Receptors and Function in Skin'.

Full details of the meeting will be circularized to members of the B.A.D. and the Investigative Group later in the autumn. Others interested should contact Professor R.M.MacKie, University Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU. The meeting is open to clinical and non-clinical colleagues.

IX Annual Conference of Indian Association of Dermatologists, Venereologists and Leprologists

The IX Annual Conference of the I.A.D.V. & L. will be held at M.G.M. Medical College, Indore (M.P.), India, from 8th to 10th January 1981.

Papers will be accepted on the above subjects. Details of registration may be obtained from: Dr N.C.Sethi, Honorary General Secretary, Manik Bhawan, Tukoganj, Indore 452001, India.

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Editor
C. D. CALNAN, M.D., London

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents.*

Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
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microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations,* A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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British Journal of Dermatology

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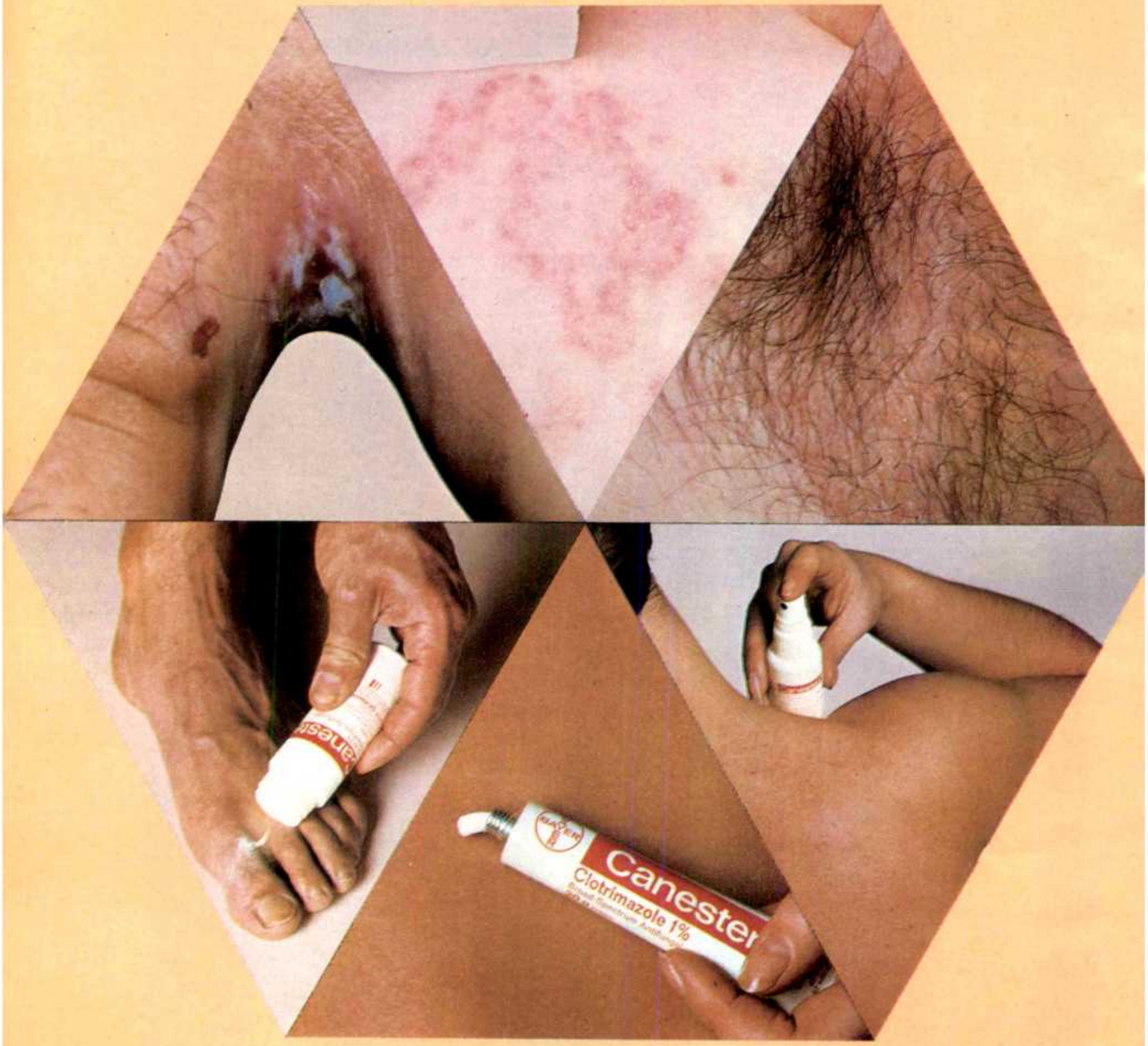

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Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost
(exclusive of VAT) £9·80
Bottle of
100 tablets (500mg)

Product Licence numbers
125mg: 4/5060
500mg: 4/5061

Glaxo

Full prescribing information on Grisovin (trade mark) is available from:
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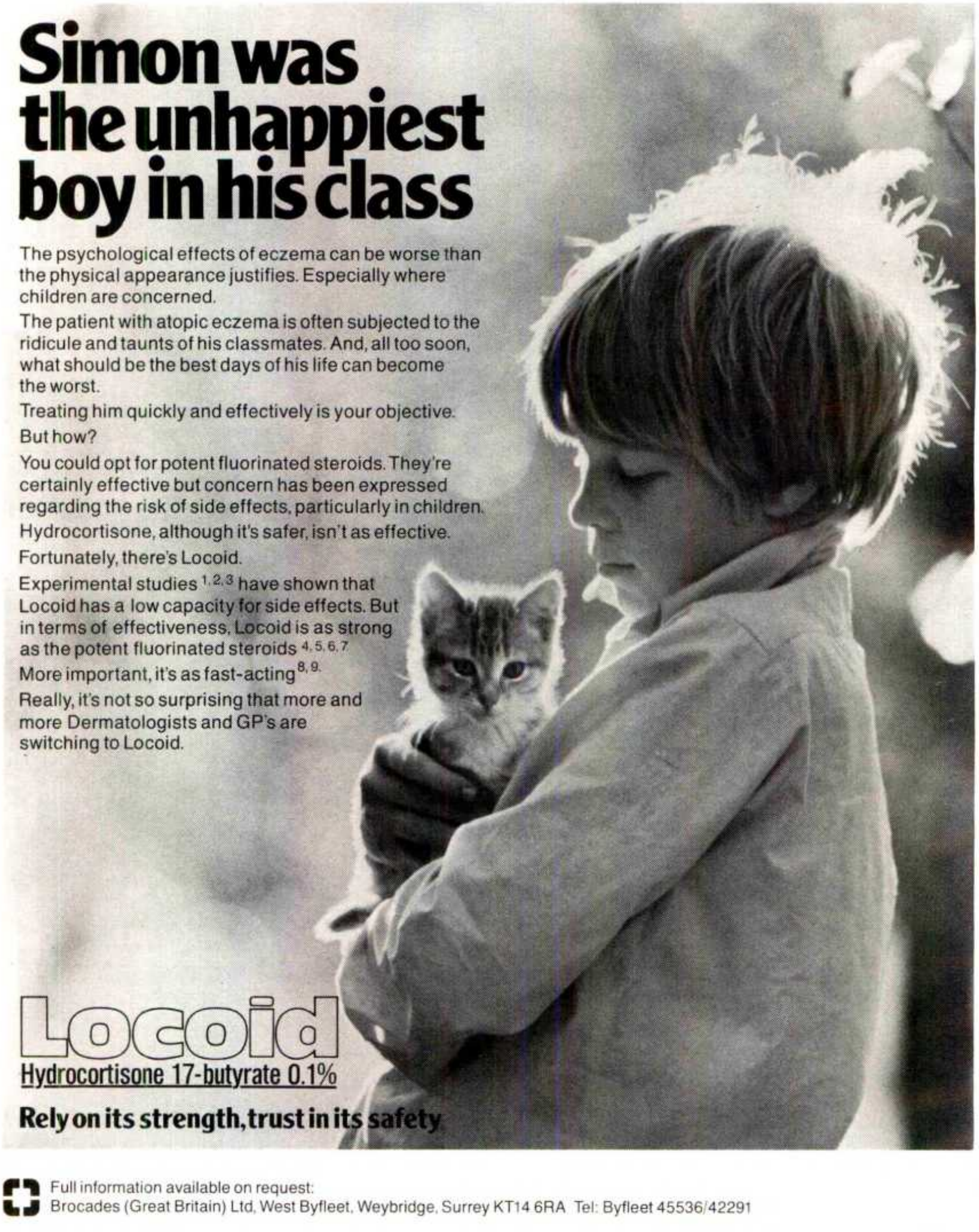

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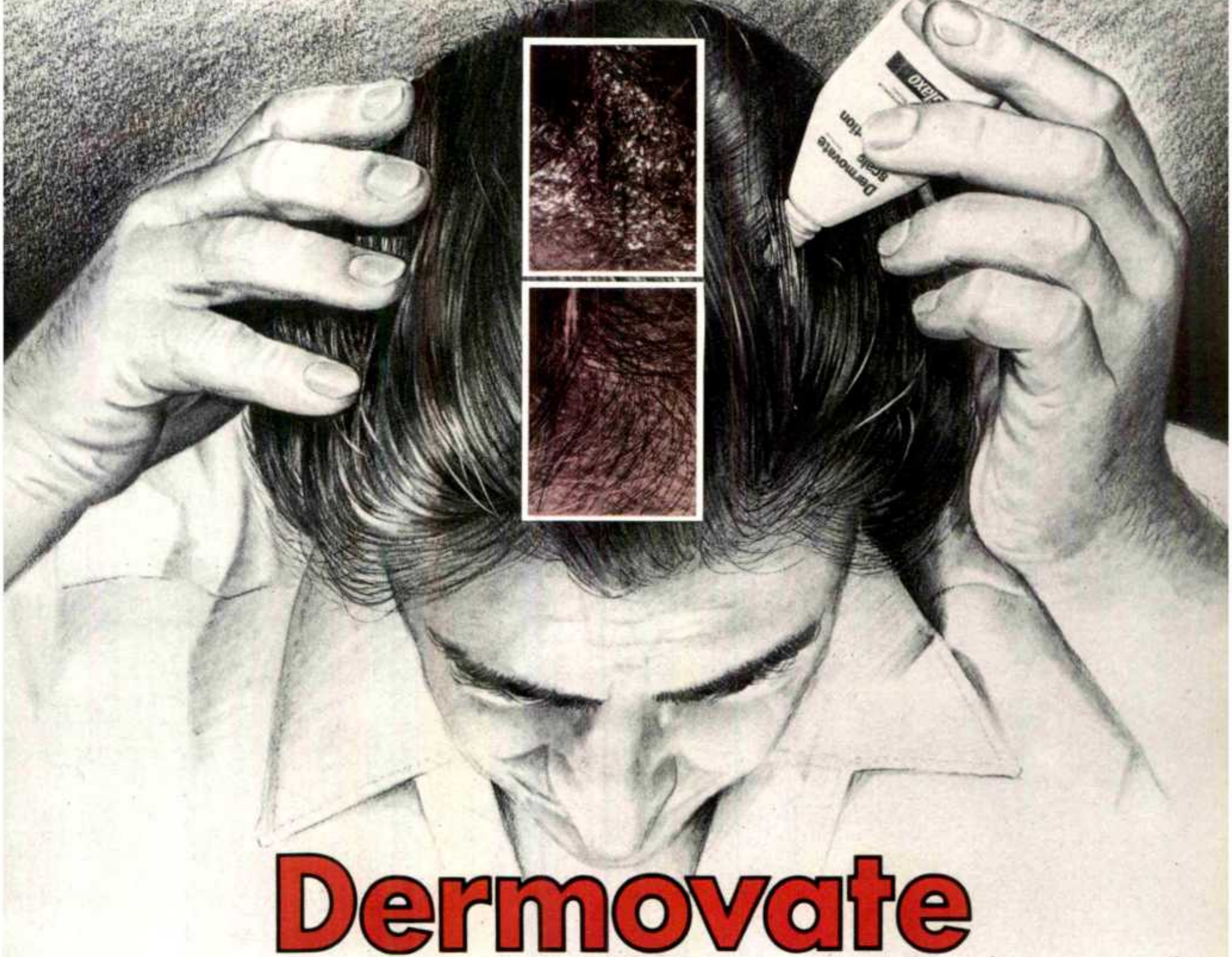

Dermovate

(clobetasol propionate)

Scalp Application

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Presentation

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100ml bottle £9·65

Product Licence number

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Side-effects
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PL No. 0085/0014
Lotion: 30 ml – £2.50
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Powder: 30 g – £2.90
PL No. 0085/0023
Spray Powder: 200 g – £2.40
PL No. 0085/0020
Spray Solution: 150 g – £3.00
PL No. 0085/0022

References:
(1) Grupper, Ch. and Avram, A. Proceedings of VIIth ISHAM Congress, Tokyo, June 1975, p. 162
(2) Schwartz, K. J. et al Deutsch. med. Wochenschr., 1975, *100,* **1497.**
(3) Gisslen, H. et al Curr. ther. Res., 1977, *21,* **681**
(4) Dorn, M. et al Munch. med. Wschr. 1975 *117,* **687**

Product Licence Holder
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*Almeyda, J. and Wood, H., (1979) British Journal of Clinical Practice, Vol 33 No. 4, 106.

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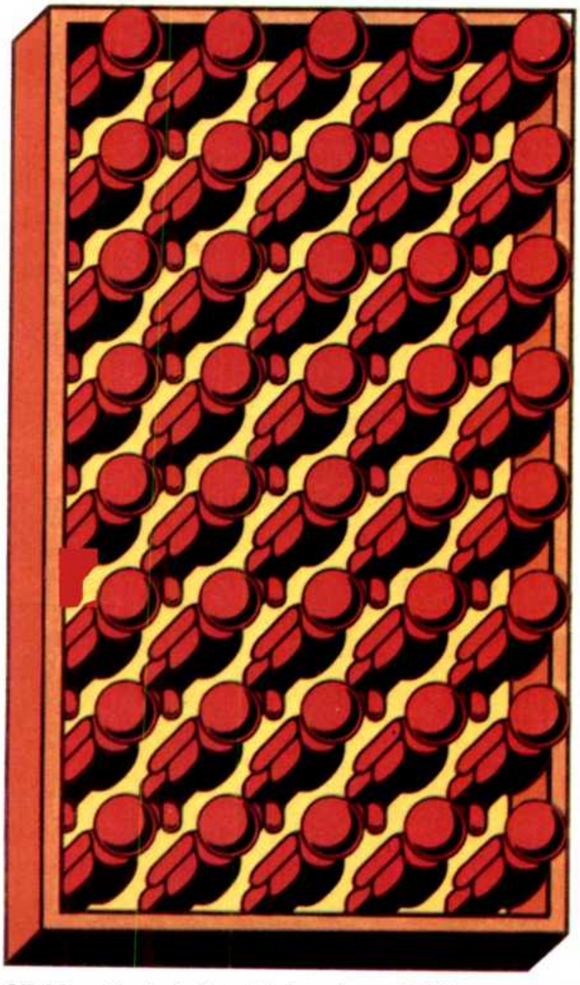

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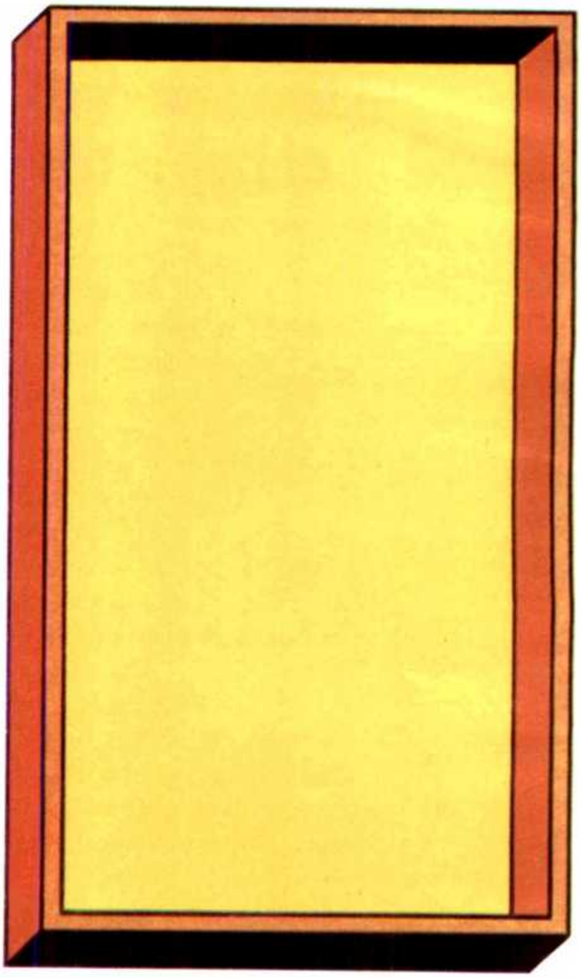

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References:
1. *British Journal of Dermatology*, 1979, **100**, 475

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British Journal of Dermatology (1980) **103**, 593.

Clinical and Laboratory Investigations

Predictive animal testing for photocontact allergenicity

TH.MAURER, E.G.WEIRICH AND R.HESS

Ciba-Geigy Ltd, CH-4002 Basel, Switzerland

Accepted for publication 20 March 1980

SUMMARY

The purpose of this investigation was to establish a standardized animal model to predict the photoallergenic potential of new chemical compounds. In a first series of experiments the main factors influencing the induction of photoallergenicity were evaluated (induction concentration, pH of the test solution, pretreatment of the irradiation site with sodium lauryl sulphate, additional use of adjuvant injections and routes of administration). Osram Ultravitalux lamps were utilized for these studies.

In a second series of experiments a Xenon 6000 W radiation source was used. Positive results were obtained with well known photosensitizers, as, for instance, the halogenated salicylanilides, as well as with so-called weak photoallergens (e.g. bithionol, sulphanilamide) by stimulating the animals' immune system with Freund's complete adjuvant.

The photoallergenic compounds were at the same time tested for their contact allergenic potential in the optimization test.

A standard procedure for the predictive photoallergenicity assay in guinea-pigs is proposed.

The use of halogenated salicylanilides as topical antimicrobial agents resulted in more than 10,000 cases of photoallergic skin eruptions in Great Britain. The high incidence of side-effects in users of soaps containing these substances led to their ban in many products (Wilkinson, 1962; Burry, 1968).

At the moment, photoallergic reactions in man are infrequent; according to Jung (1974) and Breit (1975) only 1–5% of all delayed-type hypersensitivity reactions are due to photoallergy. Further prevention is beset with difficulty because no standardized predictive animal test exists. The difficulties in detecting photoallergenic properties of drugs and other compounds were referred to recently in connection with discussions on the photoallergenicity of 6-methylcoumarin (Kaidbey & Kligman, 1978).

The method proposed by Vinson & Borselli (1966) is not a sensitive one, and is limited to the testing of soap ingredients. Other methods are more sensitive (Harber, Targovnik & Baer, 1968; Harber & Shalita, 1975; Morikawa *et al.*, 1974; Herman & Sams, 1972), but they are not standardized.

Correspondence: Dr Th. Maurer, c/o Ciba-Geigy Ltd, Toxicology GU 2.5, CH-4002 Basel, Switzerland.

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Regarding the animal model for photoallergy testing, the following elementary questions are at issue:

(1) Does induction of photoallergic reactivity in guinea-pigs depend on the concentration used?

(2) The test compounds absorb light *in vitro* from diffreent wavelengths, dependent on the vehicle and pH used. What influence does the vehicle pH have on induction and challenge?

(3) Is it necessary to stimulate the animals' immunological system with Freund's complete adjuvant, or to pre-treat their skin with sodium lauryl sulphate, to get positive results with 'weak' photoallergens?

(4) Is provocation in guinea-pigs influenced by the route of administration?

(5) Are reactions influenced by association with contact allergy?

(6) According to Schwarz (1969), UV-B light is essential for the induction of photoallergenicity in animals; what influence does UV-B have on the provocation of photoallergic reactions?

The aim of the following experiments was to answer these questions, and as the result to propose a practical method for photoallergenicity testing.

MATERIALS AND METHODS

Animals

All the tests were performed on groups of ten male and ten female guinea-pigs of the Pirbright-white strain, bred on our premises and weighing between 300 and 350 g. The animals were housed individually in Macrolon cages type 3, identified with individual ear tags, and kept at a constant room temperature of 20±1°C, at a relative humidity of 55±5%, on a 14 h light cycle day. The animals received standard guinea-pig pellets—Nafag No. 830, Gossau, SG—*ad libitum*, supplemented with fresh carrots and water.

Light sources

First test series: a row of four Osram Ultravitalux lamps (300 W each) was used (Table 1).

Second test series: A special irradiation chamber equipped with a water-cooled Xenon high pressure tube (6000 W) was used (Table 2).

TABLE 1. Light source characteristics and irradiation times. Light source: Osram Ultravitalux 300 W

	UV-B 280–320 nm	UV-A 320–400 nm	400–800 nm
Irradiation intensities at animal distance (mW/cm^2)			
Unfiltered	0·32	0·65	1·87
Filtered with window glass (2 mm)	—	0·59	1·68

Spectral range	Induction UV-B, UV-A, visible		Challenge I UV-B, UV-A, visible	Challenge II UV-A, visible
	1st	2nd and 3rd week		
Irradiation time (min)	3 (~ 1/2 MED)	6 (~ 1 MED)	3 (~ 1/2 MED)	6 (~ 1/5 MED)

Intensity measurements were done with a EG & G spectroradiometer model 585. MED (minimal erythematogenic dose) measurements were made with a sunburning UV meter from the Solar Light Company, Philadelphia.

TABLE 2. Light source characteristics and irradiation times. Light source: Xenon 6000 W Osram burner

	UV-B 280–320 nm	UV-A 320–400 nm	Visible light 400–800 nm
Irradiation intensities at animal distance (mW/cm^2)			
WG 280 (3 mm) filter	0·20	1·38	11·7
WG 320 (3 nm) filter	0·04	1·14	9·75

	Induction		Challenge I	Challenge II
	1st	2nd and 3rd week		
Filtering	WG 280	WG 280	WG 280	WG 320
Irradiation time (min)	5 (~ 1/2 MED)	10 (~ 1 MED)	5 (~ 1/2 MED)	15 (~ 1/5 MED)

Intensity measurements were done with a EG & G spectroradiometer model 585. MED (minimal erythematogenic dose) measurements were made with a sunburning UV meter from the Solar Light Company, Philadelphia.

Test compounds

TCSA (tetrachlorosalicylanilide; synthesized by Ciba-Geigy); TBS (3,5,4′-tribromosalicylanilide; K & K Lab., Inc, Plainview, NY); DBS (4′, 5-dibromosalicylanilide; synthesized by Ciba-Geigy); bithionol (2,2'-thiobis- (4, 6-dichlorphenol); synthesized by Ciba-Geigy); sulphanilamide (Fluka, DAB6, Buchs, Switzerland); Multifungin (5-bromo-4′-chlorosalicylanilide; Knoll AG, Liestal, Switzerland); chlorpromazine (active ingredient of Largactil, Megaphen, Thorazin).

Standard vehicle for all induction and challenge applications: 80% DAE433 / 20% physiological saline. (DAE433 = 40% dimethylacetamide, 30% acetone, 30% ethanol.)

Treatment

The 9 week experiments consisted of a 3 week induction period (week 1–3) followed by rest periods during weeks 4, 5 and 7, 8, and separate challenge periods during week 6 and 9 (Fig. 1). Special additional treatments are described in Results.

Induction

Four days before induction, the application site (2 × 2 cm) on the skin of the animal's neck was shaved with a hair-cutter (Aesculap 1/20 mm), and chemically depilated (5 min, Veet, Reckitt & Colman (overseas) Ltd). During the rest of the induction period, application sites were shaved daily. The animals were treated four times weekly for 3 weeks by daily applications of 0·1 ml of a 0·1% test solution. Following even distribution of the solution, the site was dried by means of an air jet. One hour later the animals were irradiated (see Tables 1 and 2 and Fig. 1).

Challenges

Four days before the first challenge week, the application site on the back of the animals was shaved and chemically depilated. During both challenges the application site was shaved daily. The test sites were treated on three consecutive days during challenge I and II with a 0·1% test solution, as during the induction period (0·1 ml per application).

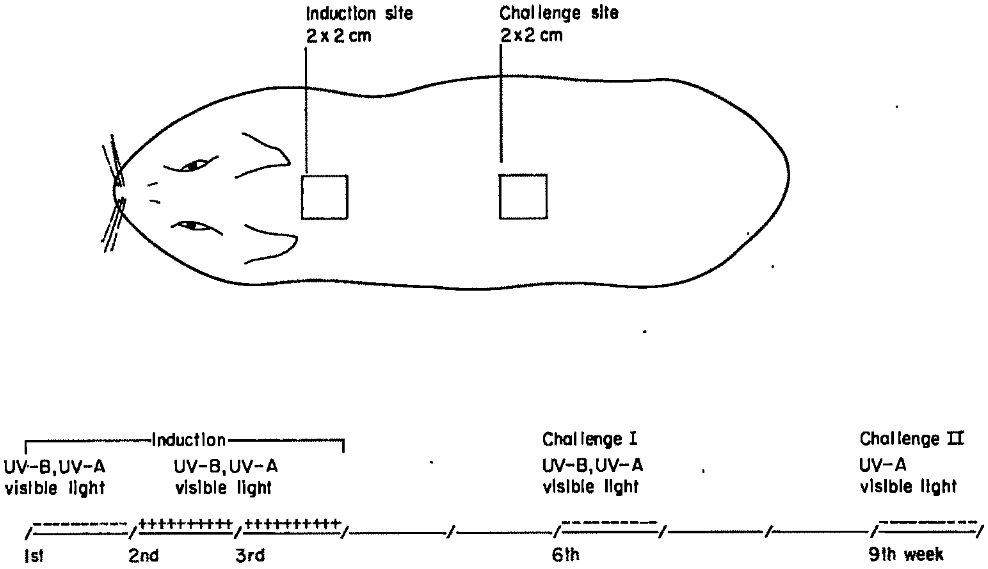

FIGURE 1. Photoallergenicity test in guinea-pigs. ––––––––––– Suberythematogenic irradiation dose (~ 1/2 MED); + + + + + + + + + + Erythematogenic irradiation dose (1 MED). Test concentration: 0·1% in 80% DAE433 / 20% physiological saline (DAE433 = 40% Dimethylacetamide, 30% Acetone, 30% Ethanol). Epidermal open application: 0·1 ml per application. Irradiation ~1 h after application.

With every test series a vehicle treated control group was included.

Irradiation (Table 1 & 2)

The animals were irradiated about 1 h after each application with:

(1) A suberythema dose during the first week of induction (UV-B + UV-A).
(2) An erythema dose during the second and third week of induction (UV-B + UV-A).
(3) Suberythema doses during challenge period I (UV-B + UV-A) and challenge period II (UV-A).

During irradiation, the animals were immobilized in a special animal holder. In order to protect the surrounding skin, it was covered with a light-proof bandage.

Evaluation of reactions

Twenty-four hours after application during the first induction week and during all challenge periods, the skin reactions were evaluated according to the Draize (1959) Scoring Scale for primary skin irritation. The skin-fold thickness was also measured at the same time with a skin-fold caliper (Oditest OOT, H.C. Kroeplin GmbH, D-6490 Schluechtern 1).

Every animal with an erythema score at the end of challenge I and/or II which was at least one score above the maximum individual erythema score of the first induction week was recorded as positive. The number of positive animals per test group was compared statistically with the number of 'pseudopositive' animals in the control group (treated with the vehicle alone).*

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* The exact Fisher test for comparison of the basic probability of two binomial distributions; L. Sachs, Statistische Auswertungsmethoden, Thieme Verlag, Stuttgart, 1975. A probability of ≤0·01 was considered to indicate a significant difference.

Evaluation of contact allergenic potentials

The animals were sensitized following the optimization procedure (Maurer *et al.*, 1978, 1979). During induction and the first challenge, 0·1% test solutions were injected intracutaneously. Six of the ten induction injections were carried out using a mixture of Freund's complete adjuvant (Difco, Michigan) and physiological saline. Subirritative doses of the test compounds were applied epicutaneously under occlusive dressings for 24 h at the second challenge. The reaction volume in individual animals after the challenge injections was compared with the threshold value after the first four induction injections. The frequency of positive animals was determined separately for the intradermal and epidermal challenges and then compared statistically with the incidence of 'positive' animals in the treated vehicle control group (see footnote on previous page).

RESULTS

Test series with Osram Ultravitalux lamps

General aspect. Examples of the results of induction and challenge reactions are shown in Fig. 2.

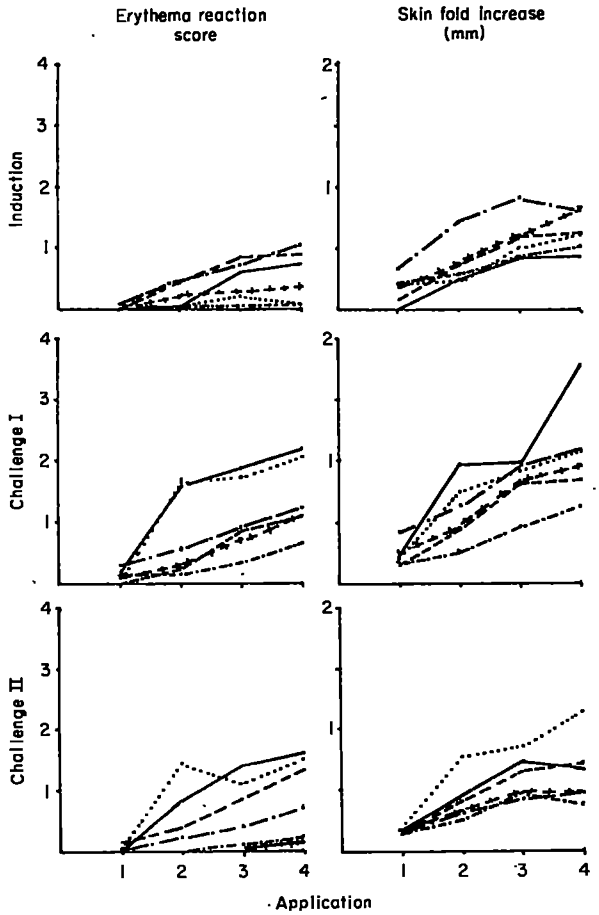

FIGURE 2. Group means of erythema reaction score and skin fold increase. ——— TCSA; - - - - - - TBS; . . . . . DBS; . -. -. -. - bithionol; . — . — . sulphanilamide; + + + + vehicle control.

Repeated irradiation with a primary suberythematogenic dose can result in a *cumulative irritative skin reaction*. The cumulative effect, due to the first challenge, was much stronger than that during induction or during the second challenge. Skin thickness increases were observed in all animals, even in those which showed no erythema after repeated irradiation with filtered light—'UV-B excluded'.

Erythema scores of 4, and deep necrotic skin injuries, were observed in the groups treated with TCSA, TBS and DBS during challenge I and II. Eschar formation was observed in all groups, and was not limited only to those animals showing strong reactions.

Comparison of control groups. The cumulative skin irritation varied from one control group to another. During challenge I (UV-A+UV-B irradiation) the incidence of positive animals was higher than during challenge II (UV-A irradiation alone). The intensity of the reactions in the control groups was in general not marked. It seems that aging of the lamp is a decisive factor.

In order to compare statistically the different influences on induction an average incidence of positive animals was used in all further tables.

Influence of induction concentration (*Table* 3). Practically all incidences after induction with 1% test solutions were decreased in comparison to those resulting from induction with 0·1% solutions. But there was no clear (inverse) relationship between doses and either reaction intensity or reaction frequency.

TABLE 3. Influence of induction concentration

	Number of positive animals per group							
	0·1% Induction concentration				1% Induction concentration			
	Challenge I	*P*	Challenge II	*P*	Challenge I	*P*	Challenge II	*P*
Vehicle control	5/20		1/20		5/20		1/20	
TCSA	16/20	<0·01	10/20	<0·01	13/20	>0·01	8/20	<0·01
TBS	8/20	>0·01	8/20	<0·01	8/20	>0·01	6/20	>0·01
DBS	19/20	<0·01	16/20	<0·01	11/20	>0·01	6/20	>0·01
Bithionol	7/20	>0·01	3/20	>0·01	2/20	>0·01	6/20	>0·01
Sulphanilamide	8/20	>0·01	4/20	>0·01	1/20	>0·01	3/20	>0·01

A phototoxic effect was shown by 1% TCSA, whereas in the other 1% groups photoprotective mechanisms were observed. Both types of 'side effects' naturally suppressed the induction of strong photoallergic reactivity in the guinea-pigs.

Influence of pH on induction and challenge (*Table* 4). According to Cripps & Enta (1970), the absorption of light by photoactive compounds in alkaline solutions results in a shift to longer wavelengths. On account of this a test series was conducted with test solutions adjusted to an alkaline pH (~pH 9) with 1-n-NaOH. With the exception of TCSA and sulphanilamide (challenge I), all groups exhibited a marked decrease in incidence following challenge I, and especially challenge II, with alkaline 0·1% test solutions than with neutral ones. The results of Vinson & Borselli (1966) were probably mainly influenced not by the pH values, but by the ingredients of the soap suspension themselves.

Influence of adjuvant injections (*Table* 4). In one test series the animals received a total of four injections of an adjuvant-saline mixture during the 2nd and 3rd induction weeks at the four corners of the application site (0·1 ml of the mixture per injection; adjuvant-saline = 1:1, adjuvant complete Freund, Difco, Michigan).

TABLE 4. Influence of pH on induction and challenge; influence of adjuvant injections

	Number of positive animals per group							
	0·1% Induction concentration: alkaline solution				0·1% Induction concentration: adjuvant injections			
	Challenge I	*P*	Challenge II	*P*	Challenge I	*P*	Challenge II	*P*
Vehicle control	5/20		1/20		5/20		1/20	
TCSA	18/20	<0·01	9/20	<0·01	19/20	<0·01	17/20	<0·01
TBS	6/20	>0·01	2/20	>0·01	14/20	<0·01	15/20	<0·01
DBS	17/20	<0·01	8/20	<0·01	19/20	<0·01	17/20	<0·01
Bithionol	4/19	>0·01	0/19		10/20	>0·01	4/20	>0·01
Sulphanilamide	9/19	>0·01	2/19	>0·01	10/20	>0·01	6/20	>0·01

The four adjuvant injections had a distinct enhancing effect on the induction in all test groups. The incidence of positive animals in all test groups and in both challenges was higher than in all other treatment groups. Stimulation with adjuvant, however, was not sufficient to produce statistically significant differences between test and control groups treated with bithionol and sulphanilamide.

Influence of SLS pretreatment on induction (*Table* 5). In one experimental series the animals were pre-treated with 0·5% sodium lauryl sulphate (SLS) before the test compound application during the 2nd and 3rd induction week.

The pre-treatment caused a higher incidence of positive animals than in the 'normal' test groups (Table 3), but the enhancing effect was less than that of the adjuvant injections.

Influence of the route of application (*Table* 5, *challenge III*). Some of the test groups were challenged a third time. The test compounds were incorporated in a mixture of 10% DAE433 in 90% physio-

TABLE 5. Influence of NaLS pretreatment and route of administration

	Number of positive animals per group					
	Challenge I	*P*	Challenge II	*P*	Challenge III	*P*
Vehicle control	5/20		1/20		1/20	
TCSA	18/20	<0·01	14/20	<0·01	6/20	>0·01
TBS	9/19	>0·01	5/19	>0·01	4/19	>0·01
DBS	17/19	<0·01	13/19	<0·01	3/19	>0·01
Bithionol	7/19	>0·01	5/19	>0·01	5/19	>0·01
Sulphanilamide	10/20	>0·01	2/20	>0·01	0/20	

0·1% Induction concentration: SLS pre-treatment during 2nd and 3rd induction week.

logical saline with 0·5% sodium stearate +1-n-NaOH (test solutions ~pH 9). This route of administration is comparable to the technique used by Vinson & Borselli (1966). The suspensions were applied for 4 h under occlusive dressings; the test sites were irradiated immediately after removing the bandages. In comparison to the second challenge without occlusion, these incidences are significantly lower in all test groups. Similar results were obtained in other treatment groups.

Open epidermal administration seems to be superior to occlusive application for 4 h in relation to the production of photoallergic reactions.

Influence of overlapping contact allergic reactions. Some of the test groups were challenged after the standard test procedure with epidermal occlusive application of the substance at a subirritative concentration for 24 h. Contact allergic reactions in photosensitized animals were observed in significant number only in the TCSA test group to which adjuvant was applied during the induction period (11/20 animals positive). All other results were negative. There is a clear-cut difference in incidence of positive animals after challenge with subsequent irradiation. It seems, especially when using weak photoallergens, that a problem with misleadingly high incidences of positive reactions due to overlapping contact allergic reactions will not arise in photoallergenicity testings.

Measurement of the increase in skin thickness. The measurement of skin thickness has the advantage of being objective. It also has the disadvantage that these measurements were increased in all test groups even when no erythematous reaction was observed. This is the reason why the differences between the various test groups were not clear-cut, and why statistically significant differences were obtained only in cases of extreme reaction intensity, but not between the control group and the test groups treated with bithionol and sulphanilamide, which exhibited only weak reactions.

Test series with the Xenon 6000 W irradiation chamber

The emission spectrum of the Osram lamps is not continuous. That could be one of the reasons why we did not get positive results with bithionol and sulphanilamide. It may be that the absorption spectrum of these compounds on the skin did not correlate sufficiently with the emission spectrum of the lamps. To exclude missing a photoactive compound, because of the 'wrong' emission spectrum of the light source, we selected from the different sources available (Magnus, 1976) the Xenon high pressure lamp for our further studies.

From the list of test compounds used in the first part, TBS was chosen as the positive reference compound, because it did not show an overlapping sensitizing potential. Bithionol and sulphanilamide were chosen as examples of weak photoallergens, which will yield positive results only in a very sensitive test system.

Positive results with *all* compounds were obtained when adjuvant was used during the 2nd and 3rd induction week (Table 6a).

To reduce the incidence of 'positive' animals in the control group, another test series was done, beginning with UV-A irradiation alone during the first induction week. The satisfactory results of the prior series were reproduced (Table 6b). Significant differences between control and test group were obtained with TBS, bithionol and sulphanilamide at challenge I and II. The erythematous reactions, however, were on the average weaker than those in the former series.

The photoallergenic potential of Multifungin and chlorpromazine as defined by our test method is comparable to that of TCSA or DBS. In contrast to the previously mentioned results of TBS, bithionol and sulphanilamide, erythematous reactions of score 4 were frequently recorded in the Multifungin and chlorpromazine groups.

TABLE 6. Incidence of positive animals per group. Light source: Xenon 6000 W. (a) 0·1% Induction concentration; four adjuvant injections during induction

	Incidence		Incidence	
	Challenge I	*P*	Challenge II	*P*
Vehicle control	4/20		1/20	
TBS	17/20	<0·01	19/20	<0·01
Bithionol	10/20	>0·01	9/20	<0·01
Sulphanilamide	6/20	>0·01	9/20	<0·01

(b) 0·1% Induction concentration; four adjuvant injections during induction

	Incidence		Incidence	
	Challenge I	*P*	Challenge II	*P*
Vehicle control	1/20		0/20	
TBS	13/20	<0·01	16/20	<0·01
Bithionol	10/20	<0·01	9/20	<0·01
Sulphanilamide	12/20	<0·01	9/20	<0·01
Multifungin	19/20	<0·01	17/20	<0·01
Chlorpromazine	16/20	<0·01	16/20	<0·01

Comparison with results from other investigators

Table 7 summarizes a few results from the literature, and from our own experiments (induction procedure including adjuvant injections during induction). The table includes the incidence of positive animals in photoallergenicity tests after challenge applications with or without irradiation.

In all instances, TCSA and chlorpromazine show the highest incidence of photoallergic as well as combined photoallergic and contact allergic reactions. In contrast to Herman & Sams (1972) and to Morikawa *et al.* (1974), we did not observe isolated contact allergic reactions with TCSA. In general, the brominated salicylanilides are less potent photoallergens than the chlorinated ones; the incidence of positive animals with combined photoallergic and contact allergic reactions is much lower too. Bithionol appears to be less active than the brominated salicylanilides.

Results of contact allergenicity tests (Table 8)

We described in one of the former sections that contact allergic reactions were observed in the TCSA test group in photoallergenicity tests. The photoactive compounds were therefore tested in a separate contact allergenicity test. With the optimization-sensitization test, statistically significant positive results for contact allergenicity were obtained with *TCSA as well as DBS*. Similar results were obtained by Chung (1977).

We conclude, therefore, that the photoallergenicity test system is not sensitive enough to examine contact allergenicity potentials in the same time. For the predictive testing of contact and photocontact potentials two separate animal models are necessary. The results for TBS in Table 8 confirm that TBS exhibits minimal contact allergenic potential, and that it is, therefore, more suitable as a positive reference compound in photoallergenicity assays than either TCSA or DBS.

TABLE 7. Contact allergenic reactions in photoallergenicity studies; incidence of positive animals per group

	Challenge reactions		
	Photoallergic reactions	Contact allergic reactions	Photoallergic + contact allergic reactions
Own experiments			
TCSA	7/20	0/20	11/20
3-4′-5-TBS	13/20	0/20	3/20
4′-5-DBS	13/20	0/20	4/20
Bithionol	3/20	1/20	3/20
Chlorpromazine	8/20	0/20	8/20
Multifungin	9/20	1/20	8/20
Herman & Sams (1972)			
TCSA	5/5	2/5	ND
3-4′-5-TBS	1/5	0/5	ND
Morikawa (1974)			
TCSA	6/24	7/24	11/24
3-4′-5-TBS	8/30	0/30	0/30
3-5-DBS	14/19	0/19	0/19
4′-5-DBS	15/36	2/36	1/36
Bithionol	2/29	0/29	0/29
Schwarz (1969)			
Chlorpromazine	3/30	0/30	15/30

ND = not done.

TABLE 8. Contact allergic potentials of photoactive compounds; optimization test

Test compound	Test concentrations		Incidence of positive animals per group	
	Challenge intradermal	Challenge epidermal	Challenge intradermal	Challenge epidermal
TCSA	0·1%	5%	11/20	19/19
3-4′-5-TBS	0·1%	3%	0/20	4/19
4′-5-DBS	0·1%	5%	7/20	20/20
Bithionol	0·1%	1%	0/20	3/19

DISCUSSION

The results of the investigations reported have shown that it is of paramount importance to combine the use of a Xenon light source with an appropriate stimulation of the animal's immune system by adjuvant injections if one wishes to obtain positive results in guinea-pigs for photoallergens which are known to be the cause of photoallergic reactions in man. Our studies have shown that the administration of solutions of test compounds at a lower concentration combined with adjuvant stimulation is superior to the use of solutions at a higher concentration alone for the induction of photoallergic reactions.

In predictive animal photoallergenicity testing it is not possible to explain how the complete photoantigen is formed in each case, i.e. whether by photoactivation of the hapten on the skin or whether by photoactivation of skin components (Storck, 1965; Pathak & Epstein, 1971; Jung, 1972). With respect to light absorption of different wavelength by the skin components and the test compounds a light source with a continuous spectrum of emission should be used. Otherwise false negative results cannot be excluded.

The percentage of contact allergic reactions occurring in the guinea-pig photoallergenicity assay is so small that it does not influence the assessment of primary photoallergic reactions. A reliable predictive evaluation of the photo- and the contact allergenic potential of various compounds in one and the same test procedure has proved to be impossible (Table 8).

In order to standardize the test method described, it will be of advantage to start the induction process with irradiations comprising only the wavelength range of UV-A (320–400 nm) and not to use a combination of UV-A *and* UV-B (280–400 nm). On the other hand, UV-B should not be excluded from the challenge procedure. This means, of course, that two challenges, one with UV-B and UV-A and the other with UV-A alone, have to be performed.

In conclusion we propose the following procedure for the predictive photoallergenicity assay in guinea-pigs.

Animal

Guinea-pig; the sensitivity of the breed and strain to the induction of photoallergic reactivity has to be gauged and regularly checked as it is done with notorious allergens in contact allergenicity tests.

Radiation source

The lamp should have a continuous emission spectrum (e.g. Xenon arc), embracing visible, UV-A and UV-B radiation.

Standard substances

Positive reference compound—TBS; standard vehicle—DAE433.

Treatment

A standard concentration of 0·1% w/w should be used for all test compounds. Pre-treatment and treatment should be performed according to the induction procedure in Fig. 1 (with combined adjuvant injections during the 2nd and 3rd induction week).

Irradiation

Irradiation should be carried out one hour after open epidermal administration of the test solutions. The following irradiation intensities should be used:

Induction, 1st week: a suberythematogenic dose of UV-A + visible light.

Induction, 2nd and 3rd week: erythematogenic dose (~ 1 MED) of UV-B+UV-A+visible light.

Challenge I: a suberythematogenic dose of UV-B+ UV-A+visible light (~1/2–3/4 MED).

Challenge II: tolerable dose of UV-A+visible light (~1/2 MED).

Assessment of the skin reactions

During the first week of induction as well as during both periods of challenge the erythema reactions are evaluated 24 hours after irradiation according to the Draize (1959) scoring scale for skin irritation. Every animal with an erythema reaction at the end of challenge I and/or II one or more score units stronger than at the end of the first induction week is registered as positive. The total number of positive animals in the test group is compared statistically with the number of positive animals of the vehicle control group according to the exact Fisher test.

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Evaluation of staining methods for resin embedded cutaneous tissue sections of mycosis fungoides

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SUMMARY

Biopsies from lesional skin of patients with histologically confirmed mycosis fungoides were embedded in resin and semi-thin ($1\mu m$) sections were submitted to a variety of staining procedures, including Toluidine blue, Richardson's, Huber, Giemsa, Paragon and Tribasic stains. The stained sections were examined with the light microscope and the quality of cellular morphological detail afforded by the different stains was assessed. The Toluidine blue, Richardson's and Paragon stains were found to be technically easy to perform and gave the best representation of nuclear and cytoplasmic detail. Of these, the authors favoured Richardson's stain. The technique of processing the tissue, and staining and mounting procedures are detailed.

The use of resin embedded material in light microscopic examination of tissue sections has allowed considerably greater definition of cellular detail than was possible with conventional paraffin embedded methods (Eastham & Essex, 1969; Green, 1970). This is of particular importance in the lymphoreticular disorders where abnormal cells can be morphologically recognized as characteristic of a specific disease (Lutzner *et al.*, 1973). The cellular preservation in resin embedded tissue is such that the examination of semi-thin ($1\mu m$) sections often obviates the need for ultrastructural study of the material (Barr *et al.*, 1978). Improvements in the embedding resins have enabled tissue to be processed rapidly (Richardson, Jarett & Finke, 1960; Chi & Smuckler, 1976) and examined using a variety of stains. This report describes a rapid method of processing skin biopsies for semi-thin sections and an evaluation of the commoner staining procedures in assessment of cutaneous mononuclear cell infiltrates.

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MATERIALS AND METHODS

Skin biopsies were obtained from representative lesions of fifteen patients with histologically confirmed mycosis fungoides. The age range was 17–71 years. There were six women and nine men.

Resin—TAAB embedding resin (TAAB Laboratories, Reading). This resin was favoured because of its short polymerization time. Mountant—Lenzol (Hopkins and Williams). Sealant—Glyceel (Hopkins and Williams).

Staining reagents

(a) *Richardson's stain* (Richardson *et al.*, 1960):
Stock solution A—1% azure II in distilled water;
Stock solution B—2% methylene blue (C.I. 52015) in distilled water;
Stock solution C—2% borax in distilled water.
The working solution is prepared on the day of use: solutions A : B : C, 2:1:1.

(b) *Toluidine Blue Stain* (Bennett & Radimska, 1966):
1 g toluidine blue O (C.I. 52040);
1 g borax;
100 ml distilled water.
The prepared stain may be stored indefinitely.

(c) *Huber Stain* (Huber, Parker & Odland, 1968):
Solution A—0·1 N NaOH;
Solution B—2% methylene blue (C.I. 52015) in distilled water;
Solution C—1 g basic fuchsin (C.I. 42510) dissolved in 20 ml distilled water at 70°C with continual stirring 2 h before use. The stain is filtered through a 0·45 μm Millipore filter before use.

(d) *Giemsa Stain* (Ruthmann, 1970):
10 ml Giemsa stain (Gurr Improved R66 solution); 90 mls 2% borax in distilled water. This is prepared on the day of use.

(e) *Paragon Stain* (Spurlock, Skinner & Kattine, 1966; Martin, Lynn & Nickey, 1966):
0·365 g toluidine blue O (C.I. 52040); 0.135 g basic fuchsin (C.I. 42510); 50 mls 30% ethanol.
The prepared stain may be stored indefinitely.

(f) *Tribasic Stain* (Grimley, Albrecht & Michelitch, 1965):
Solution A—1 g malachite-green oxalate (C.I. 42000) (Macneal), 0·4 g azure B (C.I. 52010), 1 ml aniline, 1 g phenol crystals, 100 ml 30% ethanol. Filter and store;
Solution B—1 g basic fuchsin (C.I. 42510), 25 mg distilled water.
Heat with continuous stirring at 70°C, filter through a 0·45 μm Millipore filter before use.

Technique

Elliptical skin biopsies were chopped into 2–5 mm cubes with a scalpel blade immediately after removal and fixed for 2 h in 2% glutaraldehyde at 4°C. After an overnight wash in cacodylate buffer at 4°C the specimens were post fixed in 1% osmium tetroxide for 2 h at 4°C in a fume cupboard. They were susequently washed in distilled water, dehydrated through graduated alcohols as follows: 25% ethanol 10 min, 50% ethanol 10 min, 75% ethanol 10 min, 100% ethanol 15 min, 100% ethanol 15 min, and finally washed twice for 5 min in a bath of propylene oxide. The cubes were then impregnated with resin by bathing the cubes first in a mixture of 50% propylene oxide and 50% TAAB resin for 1 h, then in two changes of TAAB resin alone for 12 h and 6 h respectively. Subsequent orientation of the specimens was facilitated by the use of a Wild stereomicroscope. The specimens were set in TAAB resin in embedding moulds with the epidermis adjacent to the cutting edge, and polymerized for 16 h at 60°C. The blocks were then trimmed and cut on a Reichert Ultracut, using glass knives.

Semi-thin 1 μm sections were placed on clean glass slides on a drop of water. Wrinkles and creases were removed by suspending a chloroform-soaked swab over the sections, which were then fixed to the slides by drying on a hot-plate. For the Huber and Giemsa stains, glass slides pre-treated with gelatin and chrome alum (0·5% gelatin and 0·05% chrome alum in distilled water) were required.

STAINING PROCEDURES

The staining techniques used in this study were all modifications of existing methods.

Richardson's stain

The slides were warmed on a hot plate to 60°C, flooded with the working solution and stained for 30 s. They were subsequently washed in distilled water, air dried and mounted.

Toluidine Blue

The slides were stained for 30 s at 70°C on a hot plate. They were then washed in distilled water, air dried and mounted.

Huber stain

The sections were stained with solution C at 70°C on a hot plate for one min. During this time the slide was covered by a glass Petri dish to diminish evaporation. The slide was washed in distilled water and the dried ring of stain removed with a razor blade. The sections were then destained with distilled water at 70°C for one min, washed with distilled water and dried in a warm air current. Subsequently the sections were covered with 2 drops of solution A and one drop of solution B at room temperature, mixed by shaking the slide for 15 s and stained for a further 15–45 s. After a final wash in distilled water, the sections were air dried and mounted.

Giemsa stain

The slides were immersed in a bath of the solution in a staining jar for 45 min at 60°C in an incubator. These were then washed in distilled water, destained in 95% ethanol for 1–3 min and then in 100% ethanol for 1–3 min. The slides were subsequently air dried and mounted. The same bath of stain could be used repeatedly.

Paragon stain

The slides were stained for 2 min at 70°C on a hot-plate covered by a glass Petri dish to diminish evaporation. They were subsequently washed in distilled water and the dried ring of stain removed with a razor blade. The slides were finally air dried and mounted.

Tribasic stain

The sections were covered with solution A for 1·5 min at room temperature, washed in a fast stream of distilled water and dried. They were then covered with solution B for 5 min, washed in distilled water, air dried and mounted.

Mounting

One drop of Lenzol was placed on a clean cover slip and the section inverted over it. With gentle pressure to the slide, all air bubbles were displaced and the cover slip could then be ringed with Glyceel. After 1 h, the Glyceel had dried to give a firm seal which could withstand even vigorous cleaning of the slides.

RESULTS

Table 1 describes the staining characteristics of the various structures seen using the different staining techniques.

Table 2 gives a summary of evaluations of the different staining techniques assessed by the authors.

TABLE 1. Staining characteristics of structures using different staining techniques

	Structure				
Stain	Collagen	Elastic tissue	Erythrocytes	Nuclear heterochromatin	Cytoplasm
Giemsa	Pale blue	Purple	Green/Blue	Dark violet	Pale violet
Huber	Blue	Purple	Dark pink	Violet	Pale pink
Paragon	Dark pink	Red	Pale pink	Dark violet	violet
Richardson's	Blue	Green/Blue	Green/Blue	Dark purple	Pale purple
Toluidine blue	Pale blue	Dark blue	Blue	Violet	Pale violet
Tribasic	Pink	Green	Green	Blue/Green	Pale green

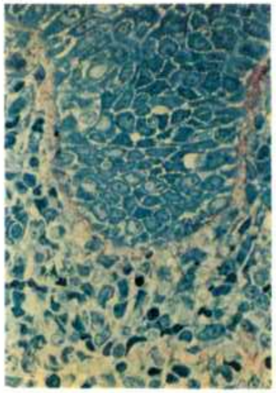
COMMENT

The use of epoxy resins as embedding media for histological material has greatly facilitated light microscopic interpretation (Jha, 1976; Barr *et al.* 1978). With improvements in both resins and technology (Glauert, Rogers & Glauert, 1956) the preparation of resin embedded material has been brought well within the scope of most histopathology departments. Resin embedding is no lengthier a process than paraffin embedding and the expense of an ultramicrotome must be counter-balanced by the excellence of the tissue preservation (Sabatini, Bensch & Barrnett, 1963). Cellular morphological detail is well demonstrated and nuclear chromatin patterns easily visualised allowing more accurate identification of different cell types. The recognition of morphologically abnormal cells may aid the more accurate classification of cutaneous infiltrative disorders and may provide a basis for the more logical management of these conditions.

Most histological stains can be used on resin embedded tissue, but the majority require initial deresination of the section (Barr *et al.*, 1978). The process of resin embedding described above is rapid, allowing specimens to be prepared and examined within 48 h and the staining procedures employed are performed on the sections still in the resin, obviating the rather lengthy deresinating procedures required for most other stains.

(Facing p. 610)

(1)



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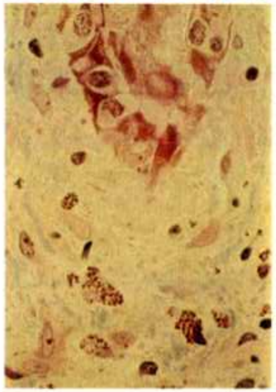

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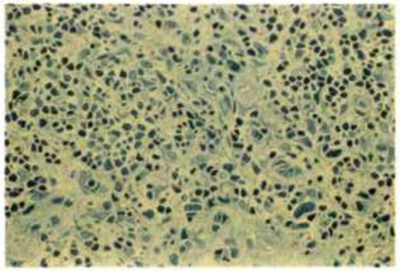

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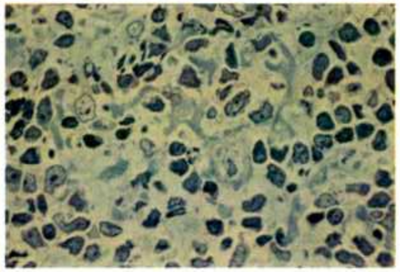

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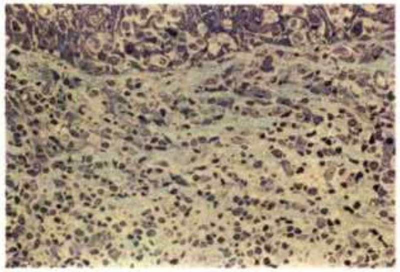

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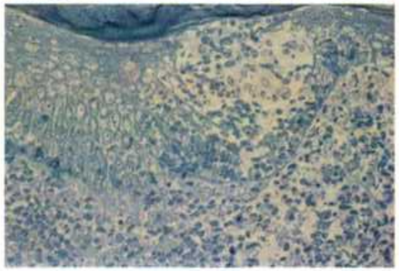

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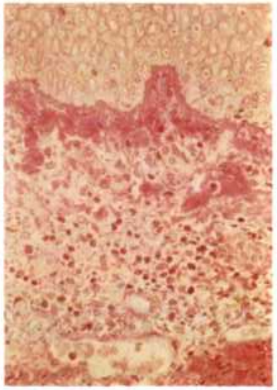

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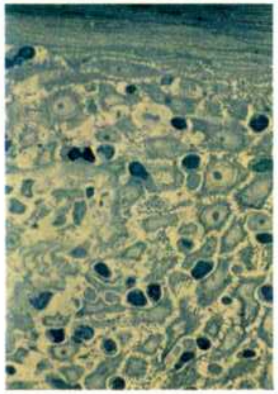

FIGURE 1. *TRIBASIC STAIN* × 300. Mycosis fungoides. Dermo-epidermal junction. Basement membrane well demonstrated as a pink band. Clear nuclear outline of histiocytes and lymphocytes in the dermal infiltrate can be seen.

FIGURE 2. *HUBER STAIN* × 500. Mycosis fungoides. Abnormal lymphoid cells seen infiltrating a hair follicle. Collagen is stained blue in the dermis and a mixed infiltrate is present.

FIGURE 3. *TOLUIDINE BLUE* × 56. Dermal infiltrate of mycosis fungoides comprising a polymorphic infiltrate of histiocytes and lymphocytes showing variable nuclear size and hyperchromatin.

FIGURE 4. *TOLUIDINE BLUE* × 500. Mycosis fungoides. Dermal infiltrate of predominantly lymphocytes, many nuclei are of irregular, indented outline and there is considerable variation in heterochromatin content.

FIGURE 5. *GIEMSA* × 56. Mycosis fungoides. Numerous abnormal cells with pale cytoplasm and cerebriform nuclei infiltrate the epidermis. A dense polymorphic infiltrate is present in the dermis

FIGURE 6. *PARAGON* × 56. mycosis fungoides. Dermal lymphohistiocyte infiltrate. Basement membrane well demonstrated.

FIGURE 7. *RICHARDSON* × 40. Mycosis fungoides. Pautrier microabscess containing mononuclear cells with cerebriform nuclei possessing variable heterochromatin. Similar cells are present in the dermal infiltrate.

FIGURE 8. *RICHARDSON* × 500. Mycosis fungoides. Individual mycosis cells are seen permeating the epidermis.

TABLE 2. Summary of observer assessment of different staining techniques

Feature	Stain					
	Giemsa	Huber	Paragon	Richardson's	Toluidine blue	Tribasic
Definition of nuclear and cytoplasmic detail	+ +	+	+ +	+ + +	+ + +	+ +
Differentiation between different cell types	+ +	+	+ +	+ + +	+ + +	+ +
Contrast between cellular and connective tissue staining	+ +	+ + +	+ + +	+ +	+ +	+ + +
Uniformity of staining	+	+	+ +	+ +	+ +	+
Reproducibility of technique	+ +	+	+	+ + +	+ + +	+ +
Comments	Often marked irregularity of staining	Reproducibility poor. Many artefacts present in sections	Basement membrane well demonstrated	Reproducible and good cellular detail	Good cellular detail.	Good contrast between cells and connective tissue

+ Poor; + + Satisfactory; + + + Good.

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In our experience, the one-step stains were technically the easiest to prepare and to perform. The Richardson's, Toludine blue and Paragon stains required only very short staining times but as a result of this, individual staining of the slides was necessary. The Giemsa stain required a much longer staining time but several slides could be stained simultaneously.

The main problem we encountered with the Giemsa stain was detachment of the sections from the glass slides during the staining procedures. This led either to loss of the section or to trapping of stain under detached areas of the section with resulting patchy staining. The problem was solved by using glass slides previously treated with gelatin and alum, but this presented its own difficulties in that completely flat sections were more difficult to achieve using these slides and the resulting section therefore frequently incorporated creases in the tissue.

The two-step stains were technically more difficult to prepare and use than the single-step stains, but the tribasic stain presented no real problems. Care needed to be exercised in preparing this stain as many of the constituents were either contact irritants or poisons and as a precaution rubber gloves were used during handling. Staining time was variable but critical as too short a time resulted in pale staining and too long resulted in a granular deposition of stain on the sections. The optimum time we found to be 1·5 min.

In contrast, the Huber stain proved to be a difficult stain with which to achieve good results. Many different factors needed to be considered when using this stain. The basic fuchsin had to be prepared 2 h before use and maintained at a constant temperature of 70°C. If the stain was allowed to cool or was heated to a higher temperature, the staining properties changed dramatically. Destaining of the sections after basic fuchsin was an essential step without which the tissue was unable to take up the methylene blue and the resulting staining was a patchy purple with no definable relationship to tissue structures. Counterstaining with alkaline methylene blue presented us again with the problem of detachment of the sections from the glass slides, and pre-treatment of slides with alum and gelatin tended to cause creases in the sections.

Results using the Richardson's, Toluidine blue and Tribasic stains were constantly reproducible and allowed detailed observation of the morphology of the infiltrating cells. Nuclear detail including the presence of nucleoli, distribution of chromatin and nuclear shape could easily be assessed, and occasional intracytoplasmic organelles could be detected.

The Giemsa and Paragon stains gave less consistent results as staining times tended to be more variable, but when successful, cellular morphology was well demonstrated. In addition, with the Paragon stain, collagen structure was well defined and the basement membrane easily detected.

The Huber stain was generally disappointing in our experience and the quality of staining was irrationally variable. Individual sections failed to take up the methylene blue even after prolonged staining times and in others the staining was so variable that no definite interpretation of results could be made.

In conclusion, the authors found that the Richardson's, Toluidine blue, Tribasic, Giemsa and Paragon stains allowed detailed observation of the cellular morphology in plastic embedded tissue sections of mycosis fungoides. For ease of use and reproducibility of results, Richardson's and Toluidine Blue stains were the most successful. The authors particularly favoured Richardson's stain and have adopted it for routine use in the assessment of cutaneous cellular infiltrates.

ACKNOWLEDGMENTS

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Cellular events leading to blister formation in bullous pemphigoid

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SUMMARY

Cellular events occurring in eight patients with bullous pemphigoid were studied by light and electron microscopy. Sections (0·5 μm) of large surface area, overlapping blisters and surrounding skin, were examined and correlated ultrastructural studies were performed on selected areas. The peroxidase contained in granules of neutrophils, eosinophils and young macrophages was visualized by incubation with diaminobenzidine and hydrogen peroxide. This cytochemical reaction was used as a marker to study the release of granule enzymes from these inflammatory cells. The release of such enzymes from eosinophils and occasionally from macrophages on the epidermal basement membrane (more precisely in the lamina lucida) was demonstrated in the skin surrounding the blisters in four patients. The release of these enzymes was also observed in the floor of the blisters in all eight patients. It is well known that these granules contain several proteolytic enzymes. These observations are therefore consistent with the proposal that proteolytic enzymes of eosinophils play a pathogenic role during the initial stages of blister formation in bullous pemphigoid.

The pathogenesis of blister formation in bullous pemphigoid is not yet well understood. The recent discovery of eosinophil chemotactic factor in the blister fluid (Baba, *et al.*, 1976) and in the serum (Czarnetzki, Kalveram & Dierksmeier, 1979) of patients suffering from pemphigoid and the observation of an accumulation of eosinophils in the superficial dermis, just before blister formation (Wintroub *et al.*, 1978), renewed interest in the pathogenicity of inflammatory cells and in the possible involvement of tissue eosinophils in blister formation.

The functions of eosinophils in tissue are not well known. As other granulocytes, eosinophils in the blood are able to perform phagocytosis and to kill bacteria (Baehner & Johnston, 1971). They contain the same lysosomal enzymes as neutrophils (West, Gelb & Rosenthal, 1975) and, as a consequence, could destroy tissue components. In spite of this, it may be thought that eosinophils are mainly antiphlogistic agents because they contain a great amount of aryl sulphatase II B which abrogates the effect of slow reacting substance of anaphylaxis (Wasserman, Goetzl & Austen, 1975),

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a histamine oxidase which destroys histamine (Zucker-Franklin, 1978), and a substance able to block the release of histamine from mast cells (Hübscher, 1975).

Using techniques reported recently (Dubertret *et al.*, 1980a, b) the present study describes the cellular events leading to blister formation in bullous pemphigoid. Morphological and functional evidence is presented for the release of granule enzymes of eosinophils along the basement membrane of the epidermis at the edge and in the floor of blisters. In this way a pathogenic role of tissue eosinophils is demonstrated.

MATERIALS AND METHODS

Eight patients with bullous pemphigoid were included in the present study. The diagnosis was proved by clinical appearance, histopathology, linear deposits of IgG and C3 on the epidermal basement membrane and the presence of epidermal anti-basement membrane auto-antibodies in serum (Table 1).

The cytochemical analysis was performed as follows: after local anaesthesia with 2% xylocaine without adrenaline 6 mm punch biopsies were taken overlapping the blister and the surrounding skin. The biopsy was placed in 10 ml of Karnovsky's fixative (Karnovsky, 1967) (prepared less than 8 days before), and diluted by half in 0·1 mol/l cacodylate buffer at pH 7·4 just before use. After 15 min of fixation the biopsy was cut into slices of 0·4 mm thickness, perpendicular to the cutaneous surface, with a newly designed hand microtome (Dubertret *et al.*, 1980a). The total duration of the fixation was 5 h at 4°C. The fragments were then washed twice for 15 min and subsequently left for 12 h at 4°C in cacodylate buffer (0·1 mol/l, pH 7·4). After fixation the fragments were incubated for 12 h in the dark at 4°C in Graham & Karnovsky medium (Graham & Karnovsky, 1966) without H_2O_2. They were incubated for 1 h in the dark at room temperature in freshly prepared Graham & Karnovsky's complete medium. This latter medium consisted of a solution of 5 ml of 3′ diaminobenzidine tetrachloride (DAB) (Sigma) in 10 ml of 0·05 mol/l Tris-HCl buffer (pH 7·6) to which 0·1 ml of freshly prepared 1% solution of H_2O_2 had been added just before use. The fragments were then washed twice for 15 min in cacodylate buffer (0·1 mol/l, pH 7·4).

Controls for the specificity of the DAB reaction were performed by incubating one adjacent fragment of the same biopsy in 2% catalase (C-100 Sigma) in 0·05 mol/l Tris-HCl buffer (pH 7·6) for 6 h at room temperature. Thereafter this fragment was put in Graham & Karnovsky medium without H_2O_2 but with 2% catalase (C-100 Sigma) and incubated for 12 h at 4°C and subsequently for 1 h at room temperature. The other adjacent fragment of the same biopsy was treated with the same protocol followed by three washes of 30 min each in 0·05 mol/l Tris-HCl buffer (pH 7·6). Then this fragment was put in a 10^{-3} mol/l $FeCN_6K_4$ solution (Merck) in 0·05 mol/l Tris-HCl buffer, shaken for half an hour at room temperature and washed three times in cacodylate buffer (0·1 mol/l, pH 7·4). The disappearance of the DAB deposition in these two controls was taken as proof that the reaction was specific for peroxidase activity.

Selective inhibition of peroxidase activity in neutrophils and monocytes was performed on tissue slices according to a modified technique described for cells in culture (Zucker-Franklin & Grusky, 1976). Tissue slices, fixed as described, were incubated in 10 ml of 0·05 mol/l Tris-HCl buffer (pH 7·6) with 6·9 mg of KCN (Merck) for 2 h with agitation at room temperature. Thereafter, they were transferred to Graham & Karnovsky medium containing the same quantity of KCN but no H_2O_2 and shaken in the dark at 4°C for 2 h. Finally, tissue slices were incubated for 1 h in the dark at room temperature in Graham & Karnovsky's complete medium with the same quantity of KCN.

Some 1 μm sections were stained as described (Dubertret *et al.*, 1980a). Some remained unstained and were examined successively by direct light microscopy and phase contrast microscopy in order to confirm the presence of DAB deposits and to localize these deposits. Photomicrography was performed with a Zeiss photomicroscope equipped with planapochromat objectives. Electron microscopy was performed on tissue sections fixed with Karnovsky fixative with or without OsO_4. Ultrathin sections were studied in a Philips 300 electron microscope.

RESULTS

In the eight patients we observed the following (Table 1).

Blisters were always in a sub-epidermal position. The epidermis showed focal necrosis of the basal keratinocytes mainly above the blister (Fig. 3). This necrosis extended in certain places into two or more cell layers. These necrosed cells appeared to be in close contact with eosinophils, macrophages or neutrophils which had migrated between the basal cells of the epidermis (Figs 1–3 and 7).

In the papillary and reticular dermis inflammatory cells were numerous but their density differed

TABLE 1. A summary of the main results from the eight cases studied

Circulating antibasement membrane antibodies	1/10	1/10	1/5000	1/1000	1/2000	1/5000	1/200	1/500
IgG linear deposits	+	0	+	+	+	+	+	0
C3 linear deposits	+	+	+	+	+	+	+	+
Blister								
Proportion of inflammatory cells in blister fluid								
eosinophils	+ +	+ + + +	+ + +	+	+ + + +	+ + + +	+ + +	+ + +
monocytes	+ + + +	+	+ + +	+ +	+	+ +	+ +	+ +
neutrophils	+	+	+ +	+ +	+	+	+	+
basophils	0	+	0	0	+	0	+	0
lymphocytes	0	0	0	0	0	0	0	0
Superficial dermis								
Proportion of inflammatory cells								
eosinophils	+ +	+ + + +	+ + +	+ +	+ + + +	+ + + +	+ + +	+ + +
monocytes	+ + + +	+	+ +	+ +	+	+ +	+	+ +
neutrophils	+	0	+ +	+	+	0	+ +	+
basophils	0	+	+	0	+	+	0	0
lymphocytes	0	0	0	0	0	0	+	+
Degranulation of mastocytes								
under the blister	+ +	+ + +	+ + +	+ +	+	+ +	+ +	+
elsewhere	+ +	+	+	+	+	+	+	0
Scattered eosinophils lysosomal granules	+ + +	+	+ +	+	+ +	+	+ +	+
Basement membrane								
Peroxidase deposit								
near to the blister	+	0	+	0	0	+	+	0
on the blister floor	+ + +	+ +	+ + +	+	+	+ +	+ +	+ +

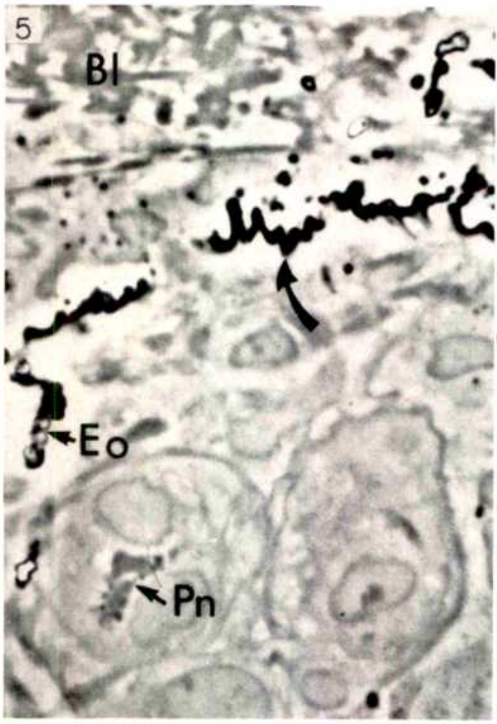
from one specimen to the other. In each biopsy the relative proportion of inflammatory cells in the superficial dermis and in the blister was the same.

Degranulation of mastocytes was always more apparent in the papillary dermis under the blister than elsewhere (Fig. 4). In this localization, subtotal degranulation was seen in two cases, degranulation of half of the granules in four cases, and degranulation was practically absent in two cases. In the papillary dermis at the edge of the blister, mastocytes were degranulated only slightly in seven cases and not at all in one case. In contrast to the findings of Wintroub *et al.* (1978), isolated granules of mastocytes were rarely observed in the dermis but some pseudogranules of purple colour resulting from collagen degradation could be confused with mastocyte granules. Mastocyte granules were never detected in eosinophils and no special relationships were noticed between eosinophils and mastocytes. Nevertheless, typical peroxidase-positive granules of eosinophils with their birefringent crystals were observed in some mastocytes of one patient.

Numerous eosinophil granules were scattered in the papillary and reticular dermis in eight cases (Figs 4 and 11). They were very numerous in four cases. Their enzymes were released among the collagen fibres (Fig. 11).

In the epidermal basement membrane zone the cellular events were as follows.

Eosinophils, and to a lesser extent macrophages, accumulated along the basement membrane, at



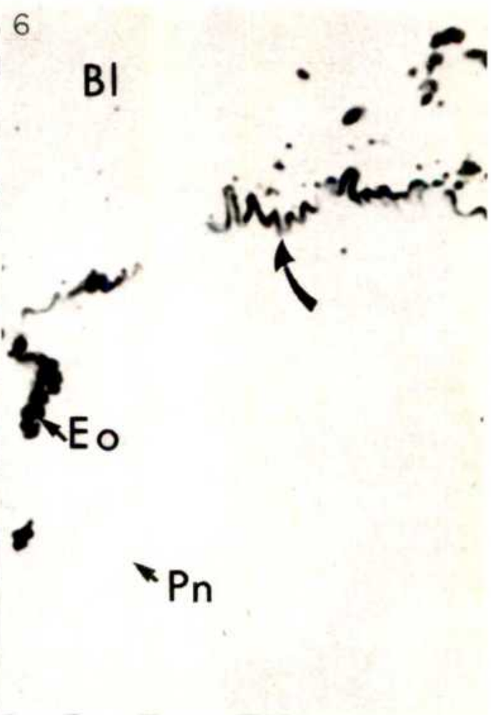
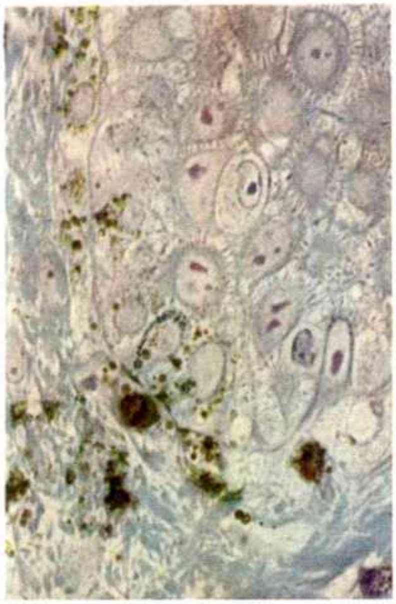


FIGURE 5. Phase contrast microscopy of a peroxidase deposit (arrow) situated on the floor of a blister (Bl). An eosinophil (Eo) may be seen at the left. A neutrophil (Pn) is inside a dermal capillary (× 2200).

FIGURE 6. Direct light microscopic examination of the same sections as shown in Figure 5. After KCN, the peroxidase of the eosinophil (Eo) itself and of the deposit (arrow) around it is not inhibited. In contrast, the peroxidase of the neutrophil (Pn) is inhibited (× 2200).

(1)



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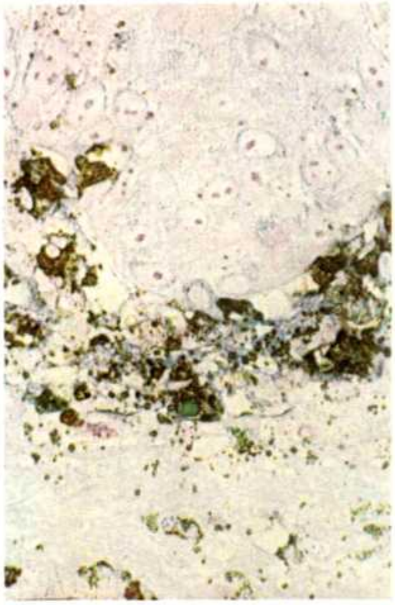

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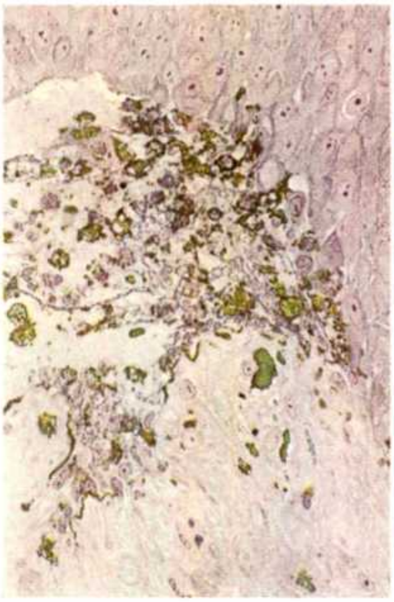

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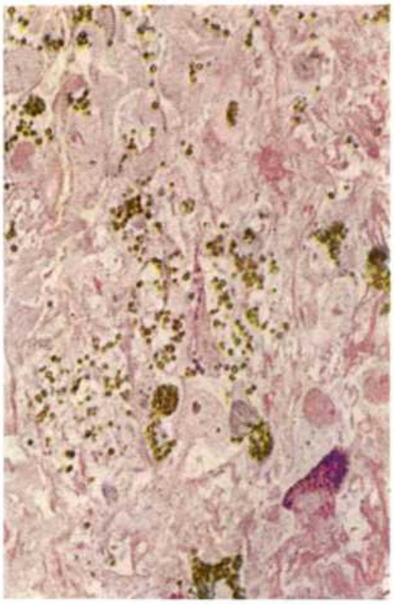

FIGURE 1. An early event: in the vicinity of an eosinophil, two small peroxidase deposits can be seen on the epidermal basement membrane. Some granules of eosinophils are scattered in the dermis and in the epidermis. The superficial dermis is vacuolized just under the basement membrane by eosinophils and macrophages. At the top left, an eosinophil has penetrated into the epidermis (× 400).

FIGURE 2. Just before blister formation: numerous inflammatory cells, mainly eosinophils, have accumulated just under the basal cells of the epidermis. Two eosinophils have penetrated the epidermis. The epidermal basement membrane has been destroyed. Two linear peroxidase deposits are situated on the papillary dermis (× 250).

FIGURE 3. A fully-formed blister: numerous eosinophils (brown cells) are contained in the blister fluid. Necrosis of the epidermal cells is limited to the basal layer and occurs in close proximity to degranulating eosinophils and macrophages. Strong peroxidase deposits are evident on the floor of the blister (× 160).

FIGURE 4. The papillary dermis: numerous brown granules of eosinophils are scattered among disorganized collagen fibres. A degranulated mastocyte is situated in the centre of the figure (× 400).

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first on the dermal side (Figs 1 and 9) but then more and more on the epidermal side (Figs 1–3 and 7). Neutrophils were less numerous and accumulated principally on the dermal side of the basement membrane.

On the dermal side of the epidermal basement membrane inflammatory cells were stretched along the basement membrane and in places had destroyed the dermal attachment of the basement membrane (Figs 1 and 9). However, this phenomenon was always very limited and anchoring fibrils were often preserved (Fig. 9). The destruction of the dermal attachment of the basement membrane was never sufficient to produce incipient blister formation.

On the epidermal side of the epidermal basement membrane progressive accumulation and degranulation of eosinophils and macrophages led to separation between the basal keratinocytes and the epidermal basement membrane (Fig. 2). In contact with inflammatory cells the epidermal basement membrane was destroyed in places. The remaining fragments were usually found on the floor of the blisters.

In all patients studied linear oxidized diaminobenzidine (DAB) deposits were observed along the epidermal basement membrane (Figs 1–3, and 5–9). These linear deposits were observed on the floor of the blisters in all cases (Figs 3, 5, 6 and 8). They were very abundant in two cases, abundant in four cases and only slight in two cases. Linear DAB deposits were also found along the epidermal basement membrane near to the blister in four patients (Figs 1, 2, 7 and 9).

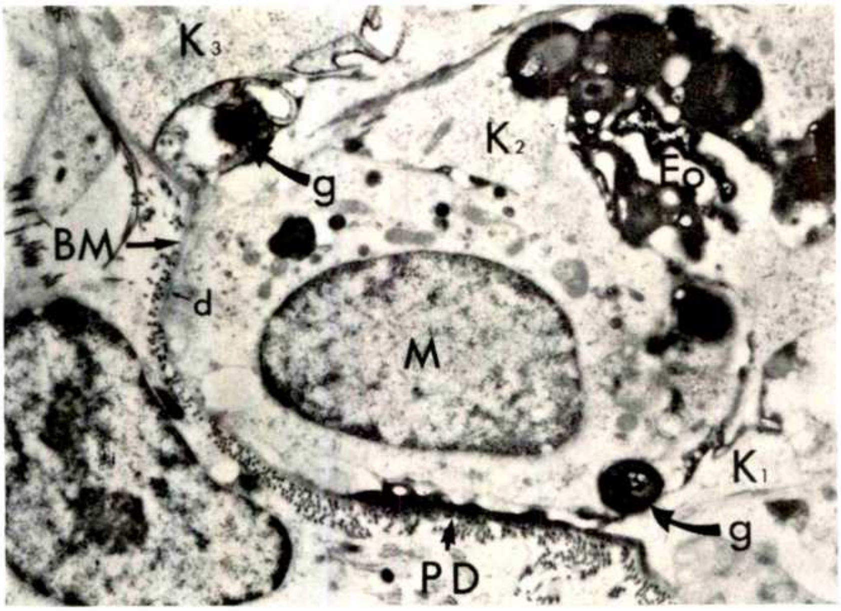


FIGURE 7. Electron microscopic examination of the peroxidase deposits seen in Fig. 1 (without OsO_4, with staining). This peroxidase deposit (PD) is situated on the basement membrane zone (BM) in close contact with the cytoplasmic membrane of the basal epidermal cell (in this case, a melanocyte (M) with an hemidesmosome (d)). This peroxidase deposit penetrates the intercellular space between the melanocyte (M) and a keratinocyte on the right (K1), and between two keratinocytes at the upper left (K2, K3). Two isolated eosinophil granules (g) are seen in the epidermal intercellular spaces and they are in process of releasing their enzymes into these spaces. An eosinophil (Eo) is degranulating in close contact with the melanocyte (M) and a keratinocyte (K2) (× 12,300).

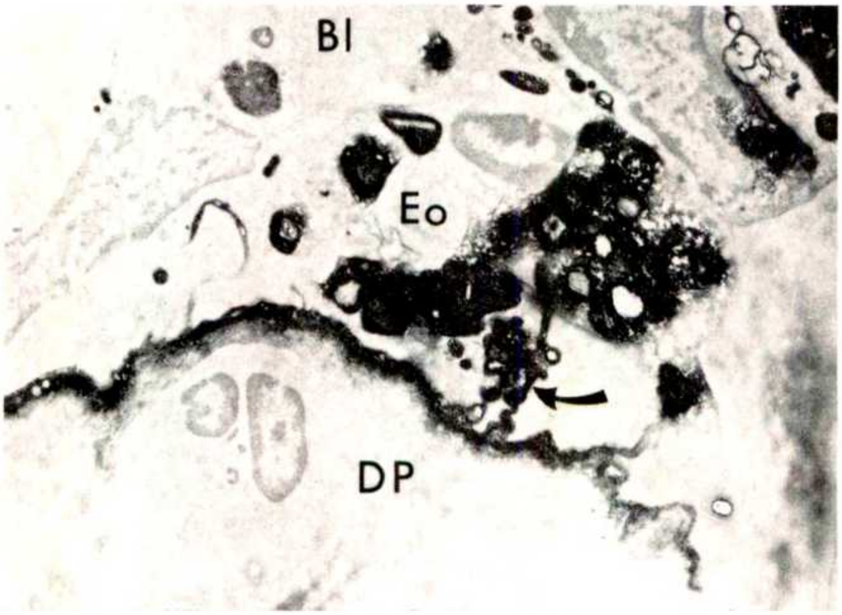


FIGURE 8. Electron microscopic examination of an eosinophil inside a blister (Bl) (with OsO4, without staining). The eosinophil (Eo) is sending a very long cytoplasmic extension containing peroxidase (arrow) towards the dermal papillae (DP) (× 9500).

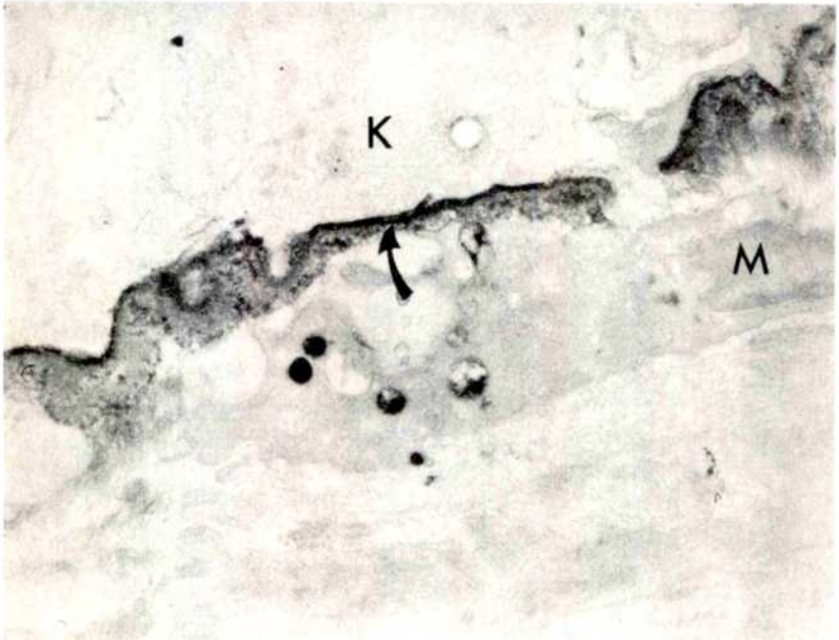
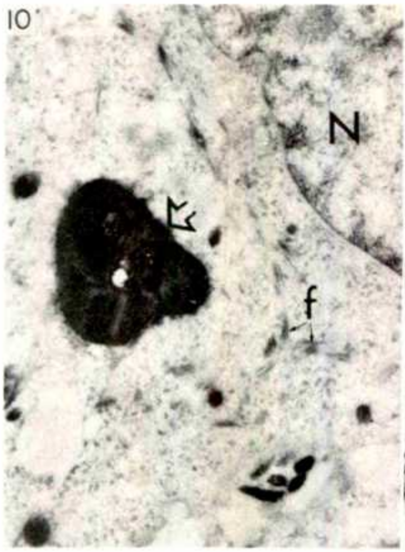


FIGURE 9. Electron microscopic examination of a macrophage in the basement membrane zone adjacent to a blister (with OsO4, without staining). A typical macrophage (M) has degranulated along the epidermal basement membrane. The peroxidase deposit is maximal in the lamina lucida (arrow) just below keratinocytes (K) (× 15,500).



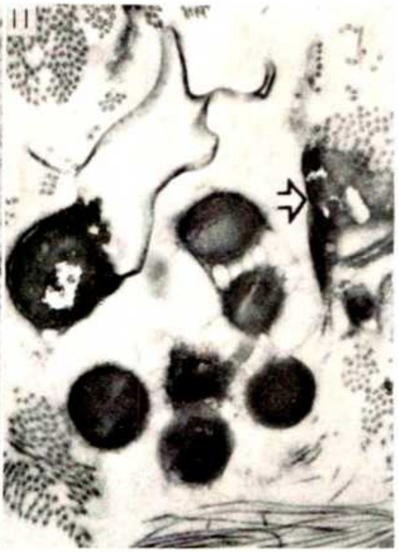


FIGURE 10. Electron microscopy showing three eosinophil granules (arrow) phagocytosed in a keratinocyte (without OsO4, with staining). Tonofilaments (f) may be seen near the nucleus (N) (× 19,000).

FIGURE 11. Electron microscopy of eosinophil granules scattered in the dermis (without OsO4, with staining). One of these granules (arrow) has released its enzymes among collagen fibres (× 19,000).

These linear deposits were oxidized DAB deposits as proved by examination of unstained sections by phase contrast microscopy and by electron microscopy (Figs 5, 8 and 9). They were the consequence of a peroxidase activity because they were not observed after inhibition by catalase and with DAB oxidized by $FeCN_6K_4$. These peroxidase deposits were not suppressed after selective inhibition by KCN of the peroxidases of macrophages and of neutrophils (Figs 5 and 6), thus it is likely that they originated mainly from the release of the peroxidase contained in the granules of eosinophils (Zucker-Franklin & Grusky, 1976).

At the ultrastructural level the peroxidase activity of the deposits situated next to the edge of blisters was maximum just between the lamina densa and the cellular membrane of basal epidermal cells in the lamina lucida (Fig. 7). This peroxidase activity, closely associated with basal keratinocytes, penetrated into the intercellular spaces between the keratinocytes (Fig. 7).

In the blisters eosinophils formed very long cytoplasmic extensions containing strong peroxidase activity and covering the dermal papillae (Fig. 8).

Degranulation of eosinophils and release of their granule enzymes around basal keratinocytes was observed in the epidermis near the blister. Isolated granules of eosinophils could be seen between keratinocytes, and some released their peroxidase content in this position (Fig. 7). Some eosinophil granules were phagocytosed by keratinocytes (Fig. 10).

Isolated eosinophil granules scattered in the dermis were demonstrated ultrastructurally. They could release their enzymes (Fig. 11) or be taken up by macrophages.

Little peroxidase deposits could be detected with light and electron microscopy in the same position as the peroxidase of eosinophils but in close vicinity to degranulating macrophages (Fig. 9). From this it is likely that enzymes originating from the granules of macrophages were released along the lamina lucida in the same way as but to a lesser extent than with eosinophils.

Release of the lysosomal enzymes of neutrophils was not observed along the basement membrane of epidermis.

DISCUSSION

Sections 1 μm thick of large surface area together with visualization of endogenous peroxidases and ultra structural observations on selected areas permitted a precise analysis of the cellular events leading to blister formation in bullous pemphigoid.

Eosinophils and, to a lesser extent, macrophages accumulated below the basement membrane of the epidermis. Thereafter, these cells migrated through the basement membrane and accumulated more and more, separating epidermal basal cells and lamina densa and leading to blister formation. During this process the content of peroxidase-positive granules of eosinophils, and occasionally of macrophages, was released along the epidermal basement membrane, or more precisely in the lamina lucida. This endogenous peroxidase does not attack tissue components but the peroxidase-positive lysosomes of eosinophils and of neutrophils contain in addition several proteolytic enzymes which may degrade tissue components (Breton-Gorius & Reyes, 1978). Thus when peroxidase is released in tissues it appears likely that lysosomal proteolytic enzymes are released at the same site. As a consequence the cytochemical visualization of endogenous peroxidase is a convenient marker for studying the release of proteolytic enzymes contained in the peroxidase positive lysosomes of eosinophils and neutrophils (Dubertret *et al.*, 1980b). It has not been established whether the peroxidase-positive granules of young macrophages contain proteolytic enzymes. Thus the release of peroxidase by a macrophage is not proof of but only a strong argument for the release of proteolytic enzymes.

The release of lysosomal enzymes from eosinophils in the lamina lucida during the initial stages of blister formation strongly suggests that these enzymes are partially responsible for this event. It may be noted that the blister in bullous pemphigoid is usually considered to form in the lamina lucida (Wilgram, 1967). It has been shown that IgG and C3 are fixed in the lamina lucida in bullous pemphigoid (Schaumburg-Lever *et al.*, 1975 and Schmidt-Ullrich *et al.*, 1975). Thus the release from eosinophils of lysosomal enzymes which may then attack the lamina lucida seems quite similar to the so-called 'immunocyto adherence' phenomenon described for neutrophils *in vitro* (Henson, 1971).

The release of enzymes from the peroxidase-positive granules of eosinophils around basal keratinocytes could be responsible for cell necrosis. This cellular aggression is interesting in view of the recently described secretion of the antigen of bullous pemphigoid by basal keratinocytes (Woodley *et al.*, 1979). A possible hypothesis is that the same antigen could attract eosinophils and macrophages in the lamina lucida and around basal keratinocytes.

The nature of the chemotactic factor leading to eosinophil accumulation remains hypothetical. In our observations, degranulation of mastocytes did not explain the accumulation of eosinophils. Selective release of lysosomal enzymes of eosinophils along the lamina lucida suggests that the chemotactic stimulus for eosinophils is maximum in this place. Thus, it could be an abnormal antigen secreted by keratinocytes, or IgG and activated C3, but also a consequence of the presence of IgE in this place (Provost & Tomasi, 1974; Atkins, Green & Zweiman, 1973).

Damage to the lamina lucida by lysosomal enzymes may induce local proteolysis and as a consequence an increase in antigenicity of the basement membrane (Remy *et al.*, 1976). In this way the disease could be maintained.

The fact that cellular events play a key role in blister formation may explain the lack of correlation between the concentration of anti-basement membrane antibody in the serum and the severity of blister formation in patients (Sams & Jordan, 1971). Many days could be necessary after the fixation of IgG and C3 in the basement membrane to attract eosinophils in sufficient quantity to induce blister formation. This delay could also explain the failure of Sams & Gleich (1971) to induce bullous pemphigoid experimentally.

In conclusion the morphological and functional cellular events leading to bulla formation in bullous pemphigoid suggest that eosinophils, and occasionally macrophages, are directly responsible for the blister formation by selective damage to the components of lamina lucida caused by the release of their proteolytic enzymes.

ACKNOWLEDGMENTS

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Isolation and characterization of tyrosinase inhibitors and their differential action on melanogenic subcellular compartments in amelanotic and melanotic melanomas

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SUMMARY

Further purification of highly active tyrosinase inhibitors by DEAE-Sephadex column chromatography has revealed that the mode of action of the inhibitors from melanomas with different melanogenic status is organelle specific. The inhibitor from amelanotic melanoma has a relatively strong suppressive effect on the tyrosinases in GERL and coated vesicle containing fractions. On the other hand, the inhibitor from melanotic melanoma has no substantial effect on these tyrosinases, although melanosomal tyrosinase is inhibited by both. The present evidence suggests that the defect of melanization in amelanotic melanoma *in vivo* is related to the tyrosinase inhibition of the smooth membrane system including GERL and coated vesicle by the highly active inhibitor characteristic of amelanotic melanoma.

Melanogenesis within melanocytes is dependent on both tyrosinase synthesis by ribosomes and the formation of premelanosomes in the Golgi area. In addition there have been a number of reports (Satoh & Mishima, 1967, 1970; Chian & Wilgram, 1967; Seiji *et al.*, 1973; Fitzpatrick *et al.*, 1979) describing the presence of melanogenic inhibitors in the pigment cells. We previously reported biochemical properties of inhibitors present in Fortner's or Greene's melanotic and amelanotic melanomas (Satoh & Mishima, 1967, 1970; Cooper & Mishima, 1967; Hamada & Mishima, 1972). They were heat stable, dialysable and did not exhibit sulphhydryl properties. In the present study, further purification and biochemical characterization of the tyrosinase inhibitors have been carried out with particular respect to their action on various melanogenic subcellular compartments of the pigment cells.

MATERIALS AND METHODS

Materials

Greene's melanotic and amelanotic melanomas originally obtained from H.S.N. Greene were used in this study.

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Preparation of crude inhibitor from tissue

Non-necrotic melanomas, excluding epidermis, were homogenized in ice-cold 0·25 M sucrose solution using an Ultra Turrax homogenizer and were further treated with a glass homogenizer chilled with ice. The suspension obtained was first centrifuged at 700 *g* for 10 min to remove unruptured cells and nuclear debris. The residue was discarded. The supernatant was further centrifuged at 12,000 *g* for 10 min to obtain a soluble fraction. The soluble fraction was treated in a dialysing apparatus using Hallow Fiber or Mini Module to obtain three different molecular weight (more than 30,000, 6000–30,000 and less than 6000) subfractions. The less than 6000 molecular weight subfraction which had the highest inhibitory activity was used as a crude inhibitor to study its chemical properties and as the starting material for the subsequent purification.

Column chromatography

DEAE-Sephadex was prepared by pretreatments with 1 N HCl and 1 N NaOH, equilibrated in 0·1 M phosphate buffer pH 6·8 and packed into a column measuring 20 × 1·5 cm. Five millilitres of the crude inhibitor was applied to the column and washed with the buffer until the absorbance of the

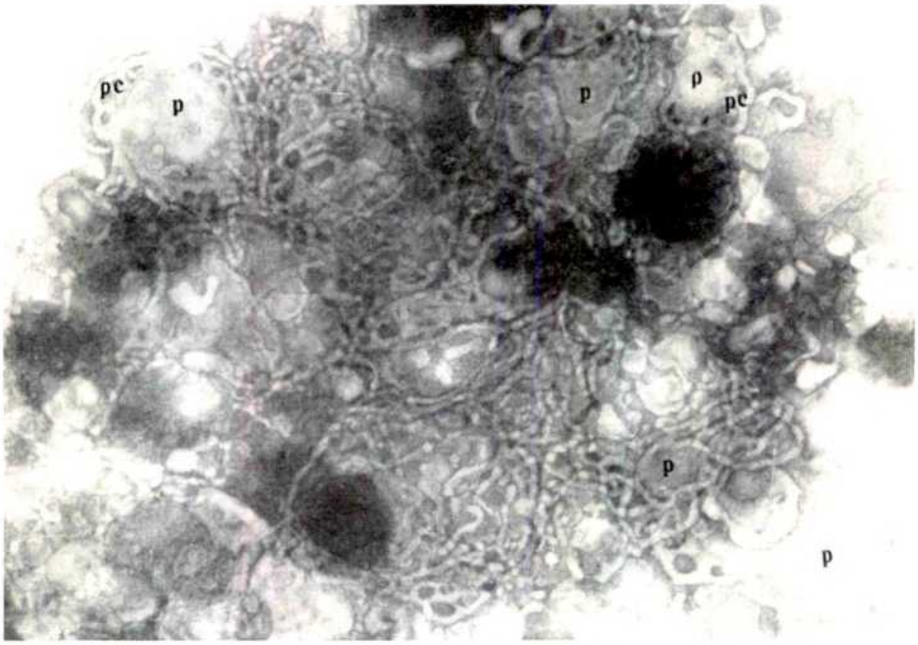

FIGURE 1. So-called Golgi fraction isolated subcellularly from Greene's hamster melanotic melanoma. Note that the Golgi apparatus is characterized by a sheet structure having polygonal compartments (pc) divided by multiple fenestrations, continuous with the plate-like central regions (p); however, this fraction is contaminated with GERL fragments composed of cisternal portion and tubular structure. It has been found previously (Imokawa & Mishima, 1978) that tyrosinase activity is present in GERL structure, but not Golgi apparatus. Negative stain with 1% phosphotungstic acid (pH 7·0) JEM-100S (80 kV) (× 33,700).

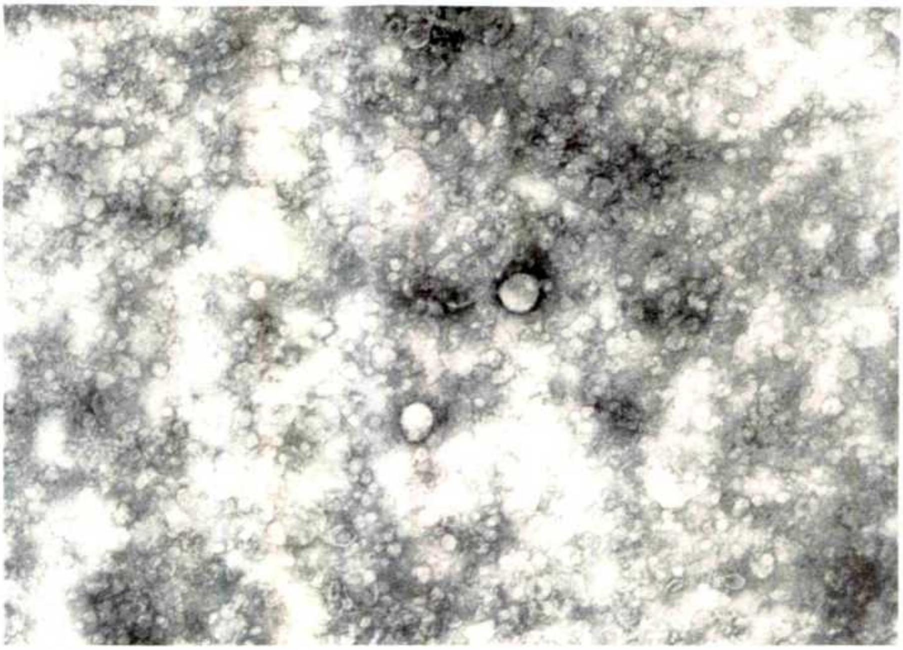

FIGURE 2. Coated vesicle fraction isolated from Greene's hamster melanotic melanoma by subcellular differential ultracentrifugation and DEAE-Sephadex column chromatography. Negative stain with 2% uranyl acetate (pH 7·0) JEM-100S(80 kV) (× 58,200).

eluent was the same as that of eluted buffer. The material was then eluted with a discontinuous gradient of 0–1·0 M NaCl. Three millilitres of the fraction was monitored at 260 nm and collected by an automatic fraction collector. Fractions belonging to each peak were combined and concentrated by freeze-drying. An appropriate volume of distilled water was added to the concentrated inhibitor powder for the inhibitor assay. As a control preparation, an equivalent volume of eluted buffer was treated in the same manner as described above to adjust the salt concentration to the same level as in the inhibitor material.

Inhibitory assay

An aqueous solution (0·2 ml) of mushroom tyrosinase (138 unit) or tyrosinase-containing subcellular fractions was mixed with 0·25 ml of inhibitor solution and 1·05 ml of 0·1 M phosphate buffer (pH 6·8) and then incubated for 1 min at 37°C, after which 1·5 ml of 2 mM dopa kept at 37°C was added to the mixture to measure immediately the initial rate of linear increase in optical density at 475 nm, based on the formation of dopachrome. The extent of inhibition by the addition of samples is expressed as a percentage or as μg protein concentration necessary for 50% inhibition ($IN_{50\%}$ μg).

Subcellular biochemistry

Fractionation of the so-called Golgi apparatus was performed by Seiji's method (Seiji, Moro &

Tomita, 1978) modified from Morre's method (Morre, 1971). Coated vesicle rich fraction was isolated by our modified method (Imokawa & Mishima, 1978) of Kadota & Kadota (1973). Electron microscopic appearances of these isolated subfractions are shown in Figs 1 and 2. Premelanosome and melanosome fractions were prepared according to the method of Seiji *et al.* (1963). Protein was determined by the procedure of Lowry, Rowebrough & Randall (1951). Enzymic properties of each subfraction used in our inhibitory assay were as follows: [Golgi fraction] Thiamine pyrophosphatase, 100–300 nmol released Pi/min/mg protein; Galactosyl transferase, 100–150 nmol transferred galactose/h/mg protein; Tyrosinase, 0·2–0·3ΔE_{475}/10 min/mg protein; Glucose-6-phosphatase, 10 nmol released Pi/min/mg protein; 5′-nucleotidase, 30 nmol released Pi/min/mg protein, [Coated vesicle fraction] Tyrosinase, 10–20 ΔE_{475}/10 min/mg protein; Adenosine triphosphatase, 300 nmol released Pi/min/mg protein, [Premelanosome fraction] Tyrosinase, 0·3–0·5 ΔE_{475}/10 min/mg protein; Acid phosphatase, 30 nmol released *p*-nitrophenol/min/mg protein, [Melanosome fraction] Tyrosinase, 0·5–0·6 ΔE_{475}/10 min/mg protein; Acid phosphatase, 20 nmol released *p*-nitrophenol/min/mg protein.

RESULTS

Isolation of crude inhibitor by dialysation

The activity of tyrosinase inhibitor is shown to be highest in the cytoplasmic soluble fractions from

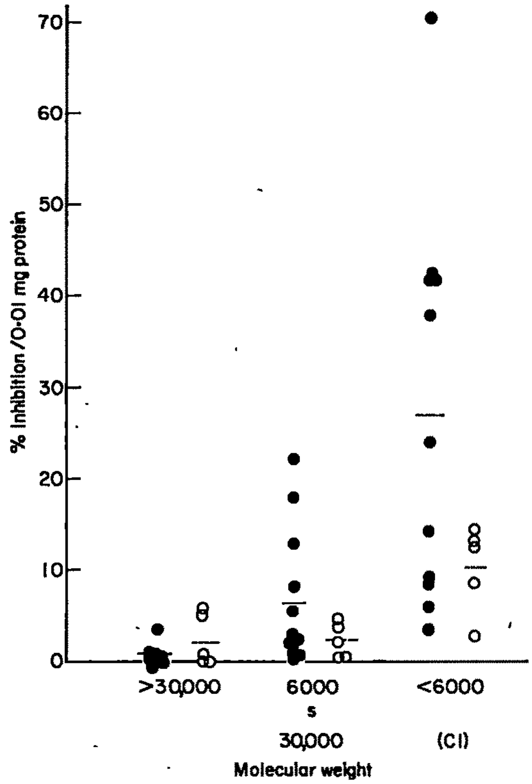

FIGURE 3. Inhibitory activity on mushroom tyrosinase of three subfractions with different molecular weights from the soluble fraction. (●) melanotic; (○) amelanotic.

TABLE 1. Inhibitory activities of crude and DEAE-Sephadex purified inhibitors on the tyrosinases in various melanogenic subcellular fractions

	Crude inhibitor					DEAE-Sephadex purified inhibitor			
	Melanotic I*		Melanotic II†	Amelanotic I‡		Melanotic II§ (Fr. C)		Amelanotic II¶ (Fr. B)	
	Inhibition (%)	Lag time (min)	Inhibition (%)	Inhibition (%)	Lag time (min)	Inhibition (%)	Lag time (min)	Inhibition (%)	Lag time (min)
Mushroom tyrosinase	55·7	0	100	33·2	0	17·3	0	21·1	0
12,000 G supernatant	41·4	1·5	—	23·1	2	11·5	0	27·5	0·75
So-called Golgi fraction	32·2	8	100	0	9	0	0	63·6	9
Coated vesicle fraction	53·5	1·5	—	24·1	5·5	0	0	58·3	2
Premelanosome fraction	58·1	3·5	100	39·1	5·5	11·9	0	43·4	0
Melanosome fraction	48·5	3	100	22·8	5·0	12·4	0	36·7	0

* 3·6 mg protein/ml; †2·96 mg protein/ml; ‡ 4·8 mg protein/ml; § 0·132 mg protein/ml; ¶ 1·7 mg protein/ml.

amelanotic and melanotic melanomas (Hamada & Mishima, 1972). In this study, this soluble fraction is first separated by the dialysation process into the three distinct molecular subfractions having molecular weights of more than 30,000 between 6000 and 30,000 and less than 6000. Inhibitory assay of the three subfractions using mushroom tyrosinase indicates the highest inhibitory activity to be present in a subfraction consisting of less than 6000 molecular weight (Fig. 3). This is used as 'crude inhibitor (CI)' for further studies. From inhibition measurements of the subfraction on subcellularly isolated melanogenic compartments containing tyrosinase, it is shown that tyrosinases in the fractions of microsome, Golgi, coated vesicle, premelanosome and melanosome are inhibited to the almost same level as mushroom tyrosinase, although there is a difference between them with respect to the presence of an initial lag time (Table 1).

Purification by column chromatography

Fractionation on DEAE-Sephadex columns results in the formation of six peaks (for melanotic) and four peaks (for amelanotic) with absorbance at 260 nm (Figs 4 and 5). Of these peaks, the third peak of melanotic melanoma and the second peak of amelanotic melanoma show the highest inhibitory activity on mushroom tyrosinase, having respectively 33 and 7 fold enrichment as compared to their crude inhibitor preparations (Figs 5 and 6). Thereafter, these peaks have been isolated and used as 'purified inhibitor' of amelanotic and melanotic melanomas respectively.

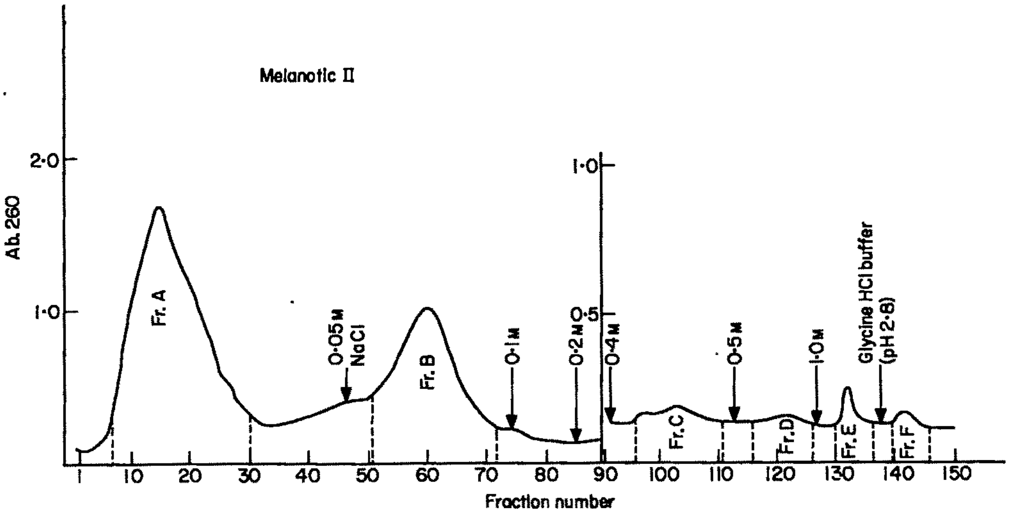

FIGURE 4. DEAE-Sephadex column chromatographic pattern of melanotic crude inhibitor.

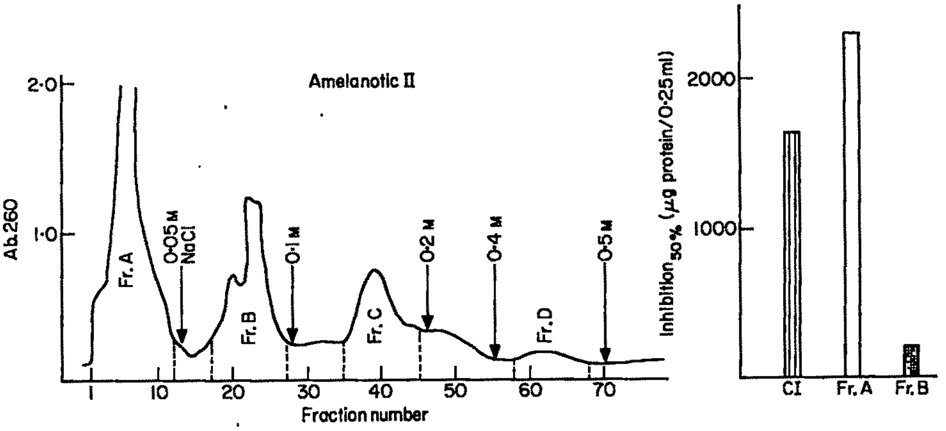

FIGURE 5. DEAE-Sephadex column chromatographic pattern of amelanotic crude inhibitor and the inhibitory activities of each peak fraction expressed as $IN_{50\%}/\mu g$.

Inhibitory activity of purified inhibitors

In contrast to the crude inhibitor fraction, the purified inhibitors of amelanotic and melanotic melanomas have been found to possess different inhibitory activities on the tyrosinases in various melanogenic subcellular compartments (Table 1). The inhibitor having the highest activity isolated from amelanotic melanoma is revealed as having a rather selective inhibitory effect on the tyrosinases in so-called Golgi and coated vesicle fractions. On the other hand, the one isolated from melanotic melanoma has no substantial inhibitory effect on these tyrosinases. However, tyrosinases in premelanosome and melanosome fractions are inhibited by both inhibitors.

DISCUSSION

There have been many reports describing the presence of melanogenic inhibitors in the cellular environment of the melanocyte, in human (Rothman, Krysa & Smiljanic, 1964), guinea-pig (Flawn

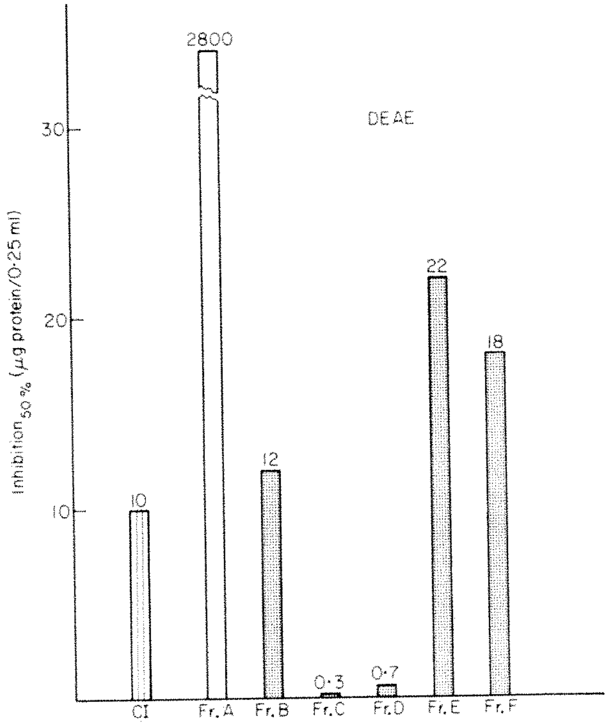

FIGURE 6. Inhibitory activities of each peak fraction obtained by DEAE-Sephadex column chromatography expressed as $IN_{50\%}$ μg (protein concentration required for 50% inhibition of mushroom tyrosinase activity).

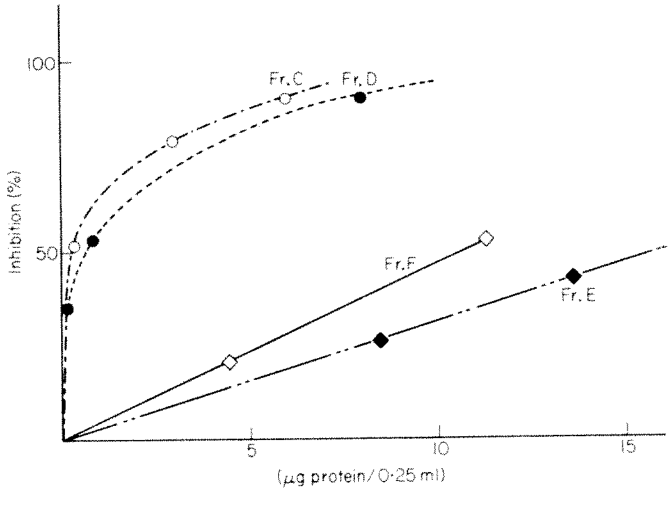

FIGURE 7. Inhibitory activities of each peak fraction obtained by DEAE-Sephadex column chromatography on mushroom tyrosinase expressed as a function of the amounts of protein.

& Wilde, 1970) and mouse epidermis (Quevedo & Isherwood, 1960) as well as in human serum (Legrand, Gonnard & Crupper, 1961).

In a previous study (Hamada & Mishima, 1972) we found a difference in the intracellular distribution of melanogenic inhibitor in amelanotic and melanotic melanomas. Amelanotic melanoma contains substantially more melanogenic inhibitor in the premelanosome fraction. As a next step, we thought that the biochemical properties of various inhibitors in different melanogenic subcellular compartments may be different and that the synthesis of each inhibitor primarily responsible for the different melanogenic status, including amelanotic melanoma (Satoh & Mishima, 1967) and albinism (Chian & Wilgram, 1967), may be characteristically altered.

The crude inhibitors of both melanotic and amelanotic melanomas which we have obtained as the molecular weight fraction less than 6000 are found to have almost the same inhibitory activity on various melanogenic membrane-associated tyrosinases (Table 1). However, the isolation of highly active inhibitors among multiple inhibitor peaks by DEAE-Sephadex column chromatography has revealed the presence of inhibitors rather specific for tyrosinases in different melanogenic organelles in malignant melanoma cells. It has been found that the purified inhibitor with the highest activity, obtained from amelanotic melanoma, has a relatively strong effect on the tyrosinase in the so-called Golgi and coated vesicle fractions. On the other hand, the purified inhibitor with the highest activity from melanotic melanoma has no substantial effect on these tyrosinases, although melanosomal tyrosinases are inhibited by both inhibitors.

Electron microscopic dopa reaction has previously revealed that not only premelanosome but also GERL and coated vesicles can synthesize dopa-melanin *in vitro*. Further, it has been suggested that tyrosinase synthesized in ribosomes is condensed and activated in GERL, and then transferred to the premelanosomes by coated vesicles budding from tubular GERL (Mishima, Imokawa & Ogura, 1979). Since tyrosinase in the so-called Golgi fraction has been found to be mostly contained in GERL but not Golgi apparatus (Imokawa & Mishima, 1978), the present findings would indicate that tyrosinase inhibited by the inhibitor from amelanotic melanoma is primarily not in Golgi but in GERL.

In amelanotic melanoma cells minimal or no melanization is present in spite of the presence of tyrosinase activity in the soluble fraction and of premelanosome synthesis. However, the mechanism of such interrupted melanogenesis has not been clarified. The present evidence would suggest that the defect of melanization in amelanotic melanoma *in vivo* is related to the rather selective inhibition of tyrosinases in the smooth membrane system, including GERL and coated vesicle, by the highly active inhibitor found from amelanotic melanoma. Further, the presence of inhibitor effective on premelanosomal and melanosomal tyrosinases in both type of melanomas may indicate the presence of a melanogenic control mechanism even in melanotic melanoma.

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Idiopathic guttate hypomelanosis

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SUMMARY

Idiopathic guttate hypomelanosis is usually associated with guttate hyperkeratosis, xerosis, and lentiginosis not related to the patient's age. Histologically, hypopigmented macules show remarkably decreased melanin, decreased DOPA-oxidase activity and a decreased number of melanosomes in the melanocytes with predominance of Stages I and II and small sizes. The epidermis is always atrophic. When scales are removed by scraping, hyperkeratotic lesions show, clinically and histologically, variable degrees of hypomelanosis; thus suggesting a relationship with the hypopigmented macules. These data suggest that idiopathic guttate hypomelanosis is the result of an early aging of the skin.

Idiopathic guttate hypomelanosis (IGH), first described by Costa (1951), was subsequently well documented in a larger number of patients by Cummings & Cottel (1966) and Whitehead, Moyer & van der Ploeg (1966).

This entity is characterized by hypomelanotic porcelain-white macules, with round or angular outlines, measuring between 3 and 6 mm in diameter, although bigger lesions have been reported. They are located mainly on the skin over the anterior aspects of the legs, forearms and trunk. The age of onset is between the third and fourth decades of life. Caucasoids and Negroids of both sexes are equally affected. The lesions of IGH are asymptomatic but they represent a cosmetic problem. Once established, they are stationary and no successful treatment is available.

Few reports (Bazex *et al.*, 1967; Argüelles-Casals & Gonzalez, 1969; Ortonne & Perrot, 1979) have been published since the original description. Although the occurrence of hypopigmented macules on the anterior aspects of the legs and usually of the forearms is characteristic, no emphasis has been made on the constant associated cutaneous abnormalities, found even in young subjects. To our knowledge, no comprehensive study of these lesions including morphological, cytochemical and ultrastructural data has been performed, with the exception of the recent work by Ortonne & Perrot (1979).

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MATERIALS AND METHODS

In an 8 month period, twelve patients consulted us for lesions of IGH, out of a total of 1419 new patients.

In seven patients, biopsies of hypopigmented macules and hyperkeratotic lesions were performed. Light microscopic examination was performed on paraffin sections stained with H & E and according to Fontana's method. Fresh samples were frozen in liquid nitrogen and cryostat sections were processed for evaluation of DOPA-oxidase activity. For ultrastructural examination, small blocks were fixed in 2·5% phosphate buffered glutaraldehyde (pH 7·3; 300 mOsm; 0·1 M) for 24 h at 4°C, washed in sucrose-phosphate buffer, postfixed in osmium tetroxide (Dalton) for 2 h, dehydrated in ethanol and propyleneoxide and embedded in Araldite. Semithin sections were stained by Richardson's method. Selected ultrathin sections were stained by uranyl acetate and lead citrate. The number of melanocytes was studied in semithin sections stained by Richardson's method.

RESULTS

Family history

A positive family history was obtained in seven patients. Family members of four of them showed both IGH and xerosis; relatives of three patients showed only xerosis. No cutaneous lesions were found in the family of one patient. No family history was available from the other four patients.

Clinical findings

Age and sex. There were eleven women, aged from 23 to 65 years, and a 33 year-old man. In most cases the reasons for seeking medical attention were aesthetic or because an infectious process was suspected. The age of onset was in the third decade in four cases, the fourth decade in three, more than 50 years in two, and unknown in the other three.

Morphological features. Guttate hyperkeratosis, xerosis, and lentiginosis, in addition to hypopigmented lesions, were constant features in all the patients, regardless of age (Figs. 1 and 2).
(a) Hypopigmented lesions: these were round, hypochromic macules measuring from 3 to 5 mm in diameter, on the anterior aspects of legs, forearms and more rarely on the trunk.
(b) Guttate hyperkeratosis: these lesions were observed in all the patients except one, irregularly intermingled with the hypopigmented lesions. Their sizes were similar to those of the hypopigmented macules. When the scales were removed by scraping, the skin beneath the scale showed normal features or a variable degree of hypopigmentation.

Incidence. The true incidence of the entity can not be estimated from our figures, as the occurrence of such lesions in patients who consulted for other dermatological problems was not recorded. An incidence of 47% out of a total of 452 in-patients who were examined systematically for the presence of this dermatosis has been reported by Cummings & Cottel (1966). An incidence of 68% in 500 unselected patients was recorded by Whitehead *et al.* (1966).

Clinical course. Our patients did not observe changes in the size of the lesions once these were established. It was difficult to state whether the number of the lesions had changed over the years, but seven patients did remark that the hypopigmented macules were more evident after sun-bathing. No subjective complaints were reported in any case.

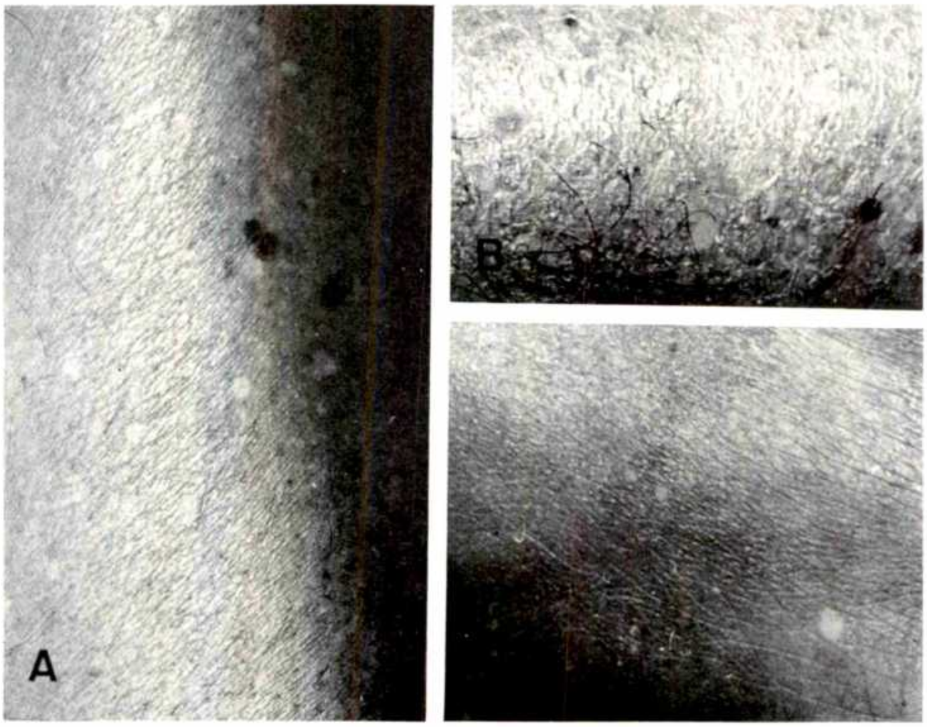

FIGURE 1. Hypopigmented macules. Note variable degrees of xerosis and the presence of lentiginosis in (A) and (B). (A) and (C) 23-year-old woman; (B) 56-year-old woman.

Laboratory studies

Direct examination and culture for fungi were negative in all cases.

Histological findings

Light microscopy observations. Hypopigmented lesions: atrophy of the epidermis was a constant feature in all cases. The horny layer was of normal thickness; lamellar in four cases and compact in the other three. No abnormalities were observed in the dermis. The melanin content in the basal layer was greatly reduced, as shown by Fontana's silver staining technique (Fig. 3). The number of melanocytes was normal.

Hyperkeratotic lesions: the epidermis was normal in all cases, but marked lamellar hyperkeratosis was always present. The cells in the granular layer had spherical nuclei and few keratohyalin granules in the cytoplasm. Using Fontana's method, a focal decrease of melanin was detected in the basal layer in three cases (Fig. 4). The pigmentation was normal in the other four. The number of melanocytes was not reduced.

DOPA-oxidase reaction. Hypopigmented lesions: a markedly reduced DOPA-oxidase activity was observed in all the cases (Fig. 5b). However, some isolated melanocytes showed a normal reaction.

Hyperkeratotic lesions: the enzyme activity was slightly reduced in some cases, normal in one, and greatly reduced in another (Fig. 5c).

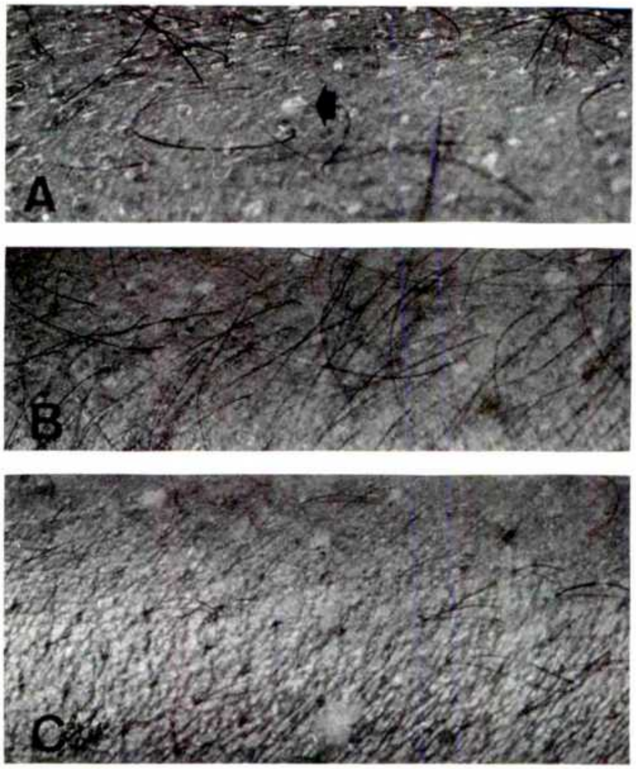

FIGURE 2. Hypopigmented lesions (arrow) in (A) a 40-year-old woman, (B) a 33-year-old man, and (C) a 26-year-old woman. Note the xerosis and the presence in (A) of marked guttate hyperkeratosis.

Ultrastructural study. Hypopigmented lesions: we did not observe a decreased number or an altered distribution of melanocytes. The cytoplasmic organelles were unremarkable. The most important feature was a decreased number of melanosomes, with a predominance of immature forms (Stages I and II). The melanosomes were small but showed a normal configuration. We did not observe clusters of melanosomes in the dendritic processes.

A decreased number of melanosomes was found in the neighbouring keratinocytes; their distribution was, however, normal.

Hyperkeratotic lesions: we did not find conclusive findings in the three cases that we examined.

DISCUSSION

Our results show that idiopathic guttate hypomelanosis (IGH) is not found as an isolated cutaneous lesion, but is always associated with guttate hyperkeratosis, lentiginosis and xerosis.

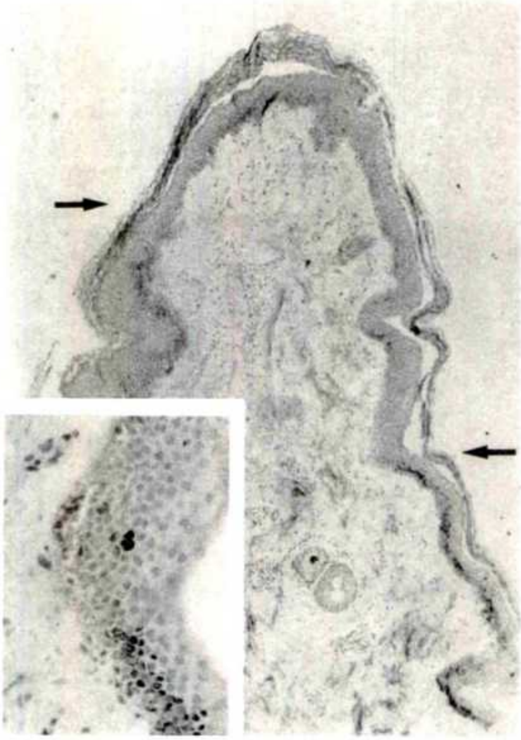

FIGURE 3. Hypopigmented lesion (arrow) showing a marked decrease in the number of melanin granules. Inset: right margin of the lesion showing sharp delimitation of the hypopigmented area (Fontana's method, × 20; inset, × 320).

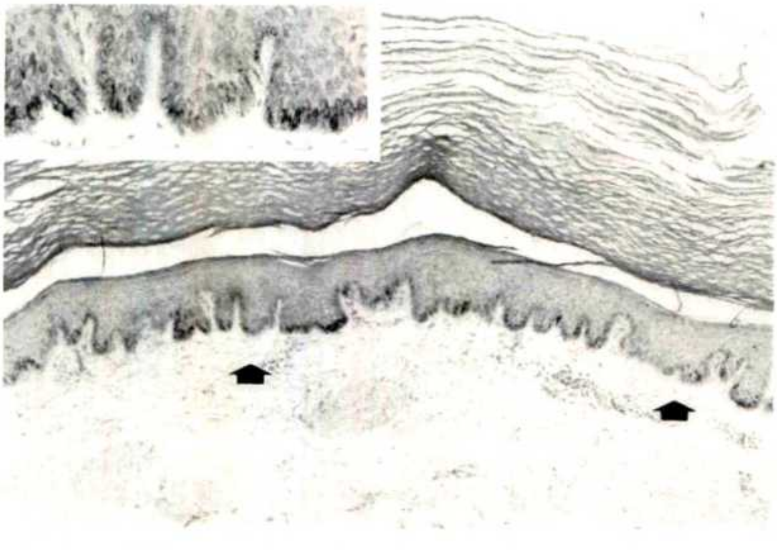

FIGURE 4. Hyperkeratotic lesion showing a focal decrease in the number of melanin granules in the basal layer (arrows). Inset: focal loss of melanin granules (Fontana's method, × 130; inset, × 320).

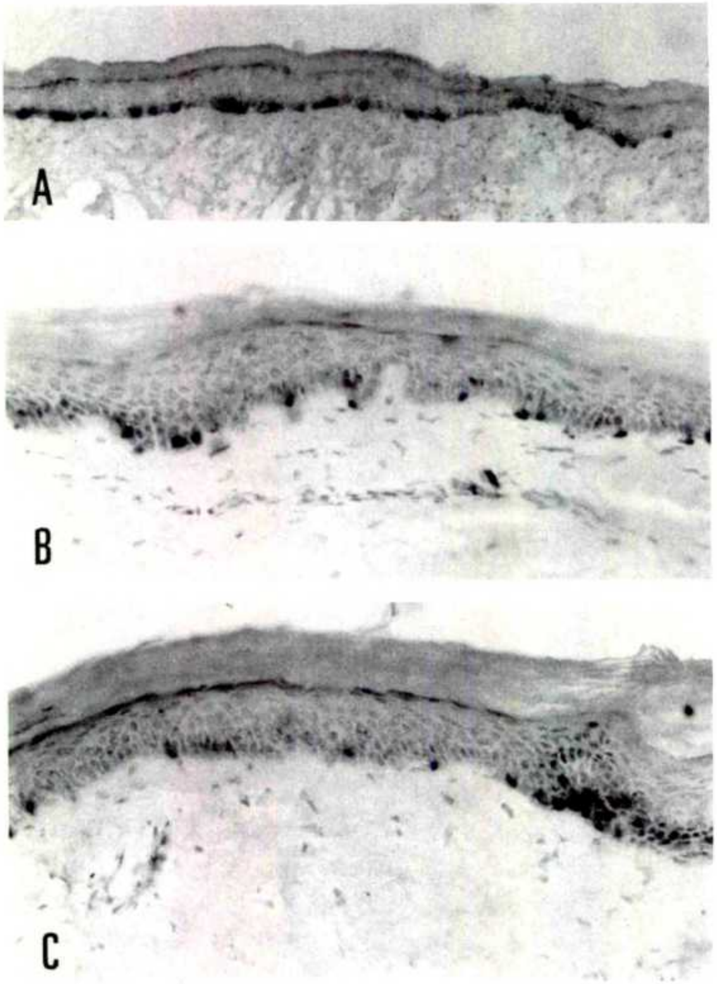

FIGURE 5. (A) Normal skin (× 50). (B) Hypopigmented lesion showing a markedly decreased DOPA-oxidase activity (× 130). (C) Hyperkeratotic lesion in which a marked decrease in DOPA-oxidase activity was observed (× 130).

In agreement with the findings of Whitehead *et al.* (1966), Cummings & Cottel (1966), Bazex *et al.* (1967), and Arguelles-Casals & Gonzalez (1969) we have found in hypopigmented lesions a decreased amount of pigment in the basal layer, decreased DOPA-oxidase activity and a normal number of melanocytes. A decreased number of melanosomes with a predominance of immature forms (Stages I and II) suggests a primary abnormality of the synthesis of melanosomes. The decrease of mature forms (Stages III and IV) and the decrease of melanosomes in keratinocytes are considered secondary changes. The decreased number of melanocytes reported by Ortonne & Perrot (1979) may be due to observing older lesions. This pattern of abnormal pigmentation is different from those described in the hypopigmented macules of tuberous sclerosis (Jimbow *et al.*, 1975), naevus depigmentosus (Jimbow *et al.*, 1975), vitiligo and piebaldism (Birbeck, Breathnach & Everall, 1961) incontinentia pigmenti achromians (Stoebner & Grosshans, 1970), hypopigmented lesions of xeroderma pigmentosum (Cesarini *et al.*, 1957), pityriasis versicolor (Charles *et al.*, 1973), leukoderma of vagabonds (Grosshans, Stoebner & Basset, 1972), and those secondary to hydroquinone and thiotepa (Jimbow *et al.*, 1974; Harben, Cooper & Rodman, 1979).

The atrophy of the epidermis is a constant feature of the hypopigmented lesions, previously unreported, but evident in the figures shown by Whitehead *et al.* (1966).

Some hyperkeratotic lesions show a focal decrease in melanin and DOPA-oxidasa activity. These facts suggest a close relationship between the hypopigmented and hyperkeratotic lesions. Although the exact mechanism remains unknown, it may be suggested that the latter precedes the former and represents a focally exaggerated xerosis.

The pathogenesis of IGH is not fully understood. The fact that the patients from Whitehead *et al.* (1966) came from sunny areas, and the predominance of lesions on exposed parts of the body, are poor criteria to conclude that IGH has an actinic origin. Our patients also came from sunny areas and their lesions were predominantly located on the anterior aspects of the legs and forearms, but no lesions were observed in other more exposed areas (face, V area of the upper chest). Moreover, some lesions could be seen in some patients on unexposed areas. We think that some of our patients' histories that the lesions were more evident during summer time probably reflects the increased pigmentation of the surrounding skin, and not a real change in their number or size.

On the other hand, no actinic degenerative changes were found in the dermis of our cases. The incidence of IGH in women who have had oophorectomy (Cummings & Cottel, 1966) is probably coincidental.

Our results suggest that IGH is an expression of an early ageing of the skin, and is always accompanied by other senile lesions, such as xerosis and lentiginosis, even in young persons. In view of the family history in our patients, we think that a genetic factor should be considered.

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Sebum levels during the first year of life*

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SUMMARY

Skin surface lipid levels in forty-five newborn babies and 193 infants aged less than 12 months were determined by an optical density method, using samples collected on ground glass. Although there was a wide individual range, sebum levels in the first week of life were very high, and were of the same magnitude as in adults. Females displayed a different pattern of sebum excretion from males. On the first day of life, the levels in females were lower than in the males but a large increase took place between the 3rd and 6th day, followed by a fall, bringing the level below that of the males. At 6 months the levels were low in both sexes. These findings suggest that there is a strong stimulation of sebum secretion before birth which probably stops at or soon after birth. Androgen may be involved in this stimulation and the probable role of dehydroepiandrosterone (DHEA) is discussed.

Very few studies have been carried out on sebum secretion in newborn children. Emanuel (1936) was the first to demonstrate that the sebum level in newborn babies can be nearly as high as in adults. The levels then gradually decline to settle at a very low baseline until puberty. Since this study, our knowledge has remained elementary, in spite of repeated allusions to a seborrhoeic pathology in the newborn. This shortcoming is probably due to the fact that the techniques available were either dangerous for the child (i.e., the ether cup method used by Emanuel (1936)), or difficult and lengthy (i.e. the extraction on filter paper used by Strauss & Pochi (1961) and Cunliffe & Shuster (1969)). The Schaefer & Kuhn-Bussius (1970) photometric method, being both harmless and quick, is free from these drawbacks and enables sebum secretion to be measured at birth.

MATERIALS AND METHODS

The method used was described by Schaefer & Kuhn-Bussius (1970). A small plate of frosted glass is applied onto the forehead for 30 s at a pressure of 1000 g. The sebum thus obtained by adsorption increases the plate's translucency in a linear way. The fall in optical density (OD) is measured with the spectrophotometer at 460 nm. The quantity of sebum obtained is proportional to the quantity present on the skin surface, i.e. to the casual level of skin lipid. The relationship between the amount of sebum collected and the number of OD units recorded with the spectrophotometer was calculated

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through a calibration curve which was found to be linear, as predicted by the definition of the OD. The calibration curve was obtained by weighing each slide before and after loading with sebum in ninety-eight randomly chosen samplings and plotting sebum weight against OD value for each slide. (Each slide had been given its own number, engraved on the side.) The calibration line was obtained by linear regression (least square method). The amount of skin lipids present on the surface of the skin was estimated to be fourfold the amount collected on the slide, according to Schaefer & Kuhn-Bussius (1970). It is known that on the forehead nearly 95% of the skin surface lipids are of sebaceous origin (Greene *et al.*, 1970). In this study every calculation was made directly from the OD. Each measurement was made from two neighbouring areas on each side of the mid-line of the forehead (Agache *et al.*, 1973). The figure obtained, the OD index, is the average of these two measurements and is expressed as optical density.

In forty-five neonates, measurements were made in the same site every day during the first week of life. They had not been washed for 12 h prior to each test, but, just after birth, the vernix caseosa had been wiped off with a piece of dry gauze. All measurements were made between 9.00 and 11.30 a.m. The age of the children was calculated from the hour of birth. None of the babies was premature, and all of them had been born without complications.

The measurements were also made at intervals in infants aged between 1 week and 1 year as follows: eighty-two children were followed from birth up to the 4th week; thirty-three children were followed to 3–4 months of age; forty-five children were followed to 4–6 months of age; thirty-five children were followed to 7–12 months of age.

The possible presence of skin lesions (acne neonatorum, seborrhoeic dermatitis, etc.) was disregarded.

Statistical evaluations were made with the t-test and the χ^2 test.

RESULTS

Sebum secretion during the first week

During the first week of life, all children showed an increase in sebum secretion (Table 1). If the recordings were expressed in optical density units (OD units vary inversely to the sebum level), we noticed that the index on the 1st day ranged from 44 to 100 OD units. On the 4th day, it was always lower than 86 OD units. Although there were wide variations between individuals, the OD decreased between the 1st and the 3rd day, and then levelled off (Table 1). This evolution was clearer in girls than in boys as shown by (i) the paired comparisons of the average daily indices, (ii) the study of the frequency of the day to day decrease of the index, and (iii) the study of index variations in the same subject from one day to the next. In the course of this evolution, one subject only, a boy, retained an index above 70 OD units up to the 5th day. The curves fell into two patterns according to whether or not the index obtained from the 1st measurement was higher than 70 OD units, i.e. whether the skin was dry or greasy. In the case of greasy skin, the curve stayed horizontal (Fig. 1). However, if the skin was dry from the first measurement (on the 1st day or at the beginning of the 2nd day), there was a rise of skin lipid with a noticeable drop in OD. These two patterns were present in both sexes, enabling us therefore to put them together for statistical analysis.

The percentage of infants with initially dry skin was 71% for the girls (seventeen cases out of twenty-four), and 43% for the boys (nine cases out of twenty-one) but this difference was not significant. On the 1st day, the difference between the average of girls and boys was significant. On the following days, the post-natal secretion of sebum was greater in the girls. On the 4th, 5th, and 6th days, the difference between the sexes was not significant for each day taken separately, but became significant if the samples of 3 days were put together (Fig. 2).

TABLE 1. Average daily OD indices. Evolution according to sex in the first week of life. Note that OD index is inversely related to sebum excretion rate

Day after birth	1st	2nd	3rd	4th	5th	6th	7th
Females							
Mean index	81·2	69·0	64·2	58·8	57·4	56·6	62·2
S.d.	12·5	15·4	13·8	10·5	10·0	11·6	17·7
N	13	24	24	22	22	19	8
Males							
Mean index	68·2	65·2	60·6	60·2	64·7	58·2	54·7
S.d.	14·7	13·8	11·3	11·3	10·9	9·4	7·4
N	13	20	21	21	16	13	10

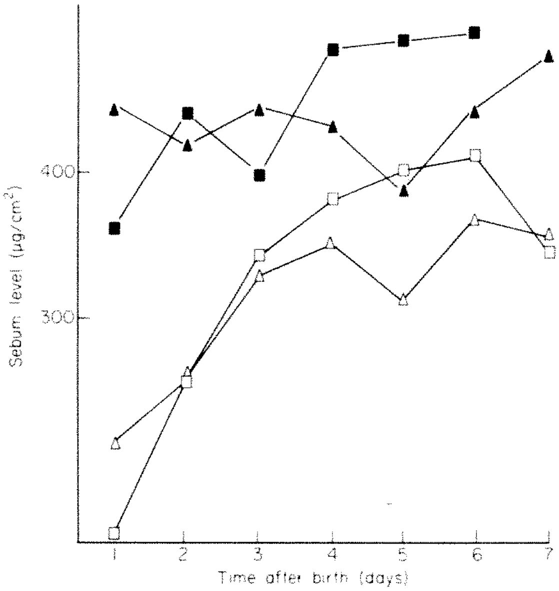

FIGURE 1. Average casual levels of sebum during the first week of life. (■—■) Females, greasy skin (>400); (▲—▲) males, greasy skin (>400); (□—□) females, dry skin (<300); (△—△) males, dry skin (<300).

Sebum secretion during the first year

At the end of the first week, there was a slight decrease in the girls' sebum level, statistically significant when compared with the 4th, 5th, 6th day levels. During the first month, the average sebum level in girls was below that in boys, but the difference was not statistically significant (Table 2). During the 2nd and 3rd months, the level fell gradually to the 6 month baseline (Table 3, Fig. 3) remaining there until the end of the 1st year of life. No difference was found between sexes. The change in sebum secretion was statistically significant between the 1st month and the 2nd quarter of the year, but not between the 2nd quarter and the next 6 months.

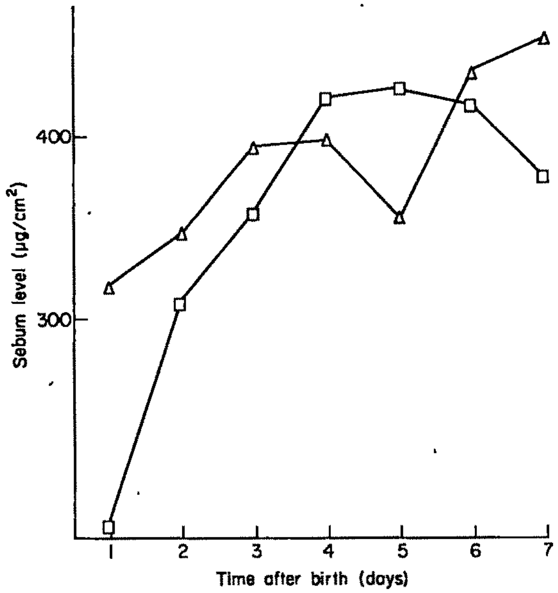

FIGURE 2. Average casual levels of sebum during the first week of life according to sex, and whether the skin was dry or greasy on the first day. (□—□) Females; (Δ—Δ) males.

TABLE 2. Average weekly OD indices. Evolution throughout the first month of life. Note that OD index is inversely related to sebum excretion rate

	1st week (Day 2+3+4+5)	2nd week	3rd week	4th week
Females				
Mean index	62·5	57·2	70·7	73·0
S.d.	10·8	11·5	23·7	3·5
N	22	11	3	2
Males				
Mean index	61·3	57·4	63·4	72·0
S.d.	10·7	12·4	20·0	
N	23	10	7	1

TABLE 3. Average OD indices. Evolution throughout the first year of life. Note that OD index is inversely related to sebum excretion rate

	1st week (Day 2+3+4+5)	Week (2+3)	Month (2+3)	Month (4+5+6)	Month (7–12)
Females					
Mean index	62·5	61·7	72·0	88·8	84·9
S.d.	10·8	14·7	14·8	7·7	11·6
N	22	16	16	21	14
Males					
Mean index	61·3	60·6	72·4	83·7	90·0
S.d.	10·7	15·5	9·5	13·1	10·4
N	23	18	17	24	19

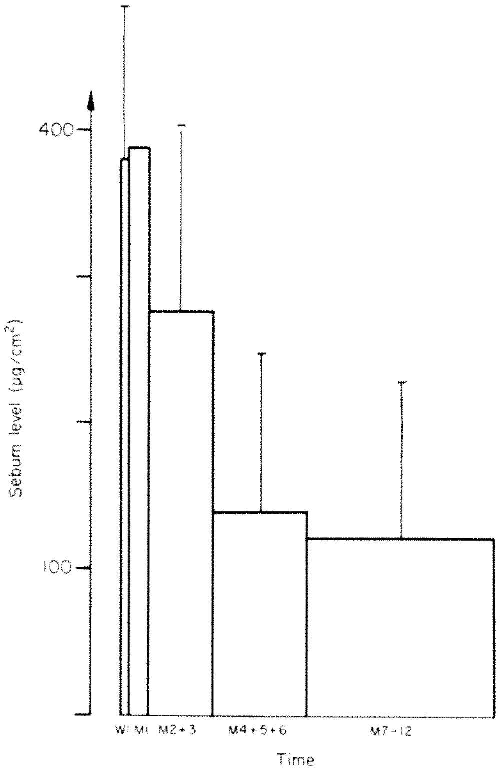

FIGURE 3. Average casual levels of sebum during the first year of life. Bars indicate s.d. of data. W = week; M = month.

DISCUSSION

Emanuel (1936) has shown that there is a marked post-natal sebum secretion. Our findings revealed that the levels obtained on the 4th day of life were comparable to those of young adults (Table 4). This is an especially interesting fact which should be emphasized. In boys, a high level of sebum secretion was often detectable from birth with a slight post-natal increase, and the level remained fairly constant during the whole of the 1st month (Table 2). In girls, however, the sebum levels were rather low on the 1st day, rose higher than in boys on the 5th day, and decreased around the 8th to a level slightly below that of the boys. From the 2nd month onward, there was a notable decrease in sebum secretion in both sexes, to the end of the 1st year of life.

Just after birth, there occurs a 'genital crisis' characterized by breast swelling, sometimes with milk production, oedema of the genitalia with hydrocoele in the male, and genital haemorrhages in the female (Lelong, 1962). These changes, which are found in both sexes, last only 2 or 3 days. We have also observed some acne lesions and/or a male pattern alopecia up to Hamilton's Type IV occurring in both sexes a few weeks later, and spontaneously subsiding by the 3rd or 4th month.

The post-natal sebum secretion and the genital crisis take place at the same time, but the former lasts much longer and seems related to the skin conditions which occur more usually after puberty, namely male pattern alopecia, seborrhoeic dermatitis (during the course of which we noticed a higher level of sebum) and acne.

Epstein & Epstein (1966), using *in vivo* labelling with thymidine-H3, found a renewal time of 7·4 days for human sebaceous glands. Downing *et al.* (1975) showed in adults that it takes a week

TABLE 4. Comparison of forehead average OD index in 4-day-old babies and young adults without acne. Note that OD index is inversely related to sebum excretion rate

		Females	Males
Newborn babies: 4th day of life	Mean	58·8	60·2
	S.e. mean	2·2	2·5
	N	22	21
Adults: 15–40 years	Mean	64·3	62·9
	S.e. mean	2·2	2·2
	N	51	28

between the incorporation of ^{14}C labelled acetate in the gland and the appearance of labelled sebum on the skin surface (i.e. from formation to excretion), but Plewig & Christophers (1974) suggested 3 weeks, the 7 days only representing the excretion time of cells which can incorporate ^{14}C labelled acetate for lipogenesis.

On this basis, even if the transit time is shorter in neonates because of the high degree of metabolic activity and the thinness of the skin, our findings would mean that the androgenic stimulus responsible for the sebum secretion has taken place before birth.

These changes in sebum secretion should also be compared with the rates of circulating androgens. In the first day of life, Forest & Cathiard (1975) and Forest *et al.* (1973b) found high blood rates of free testosterone and 4 androstenedione. In girls, these rates drop in 2 weeks to the prepubertal level. In boys, after a short decreasing phase, the rates rise again, peak by the 3rd month, and then slowly decrease to the prepubertal level by the 7th month (Brooks, 1975; Forest & Cathiard, 1975; Forest *et al.*, 1973a). Both these patterns differ from that of the sebum level, and this makes it unlikely that these two hormones are the main stimulus to sebum secretion. On the other hand, the plasma levels of dehydroepiandrosterone (DHEA) seem to be a plausible sebum stimulus, because they are elevated at birth (by 10 – 15 times the levels of other androgens) in both sexes equally and they gradually decrease during the first 3 months of life (de Peretti & Forest, 1976).

The higher percentage of greasy skins found at birth in boys could be explained by the higher levels of free plasma testosterone found in newborn males by Forest *et al.* (1973a, b) if the assumption was made that testosterone would act on only DHEA-activated sebaceous glands. Such an assumption would also explain the lack of a rise in sebum levels by the 3rd month of life while plasma testosterone levels are peaking.

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Delayed skin test reactivity to *Propionibacterium acnes* correlates with severity of inflammation in acne vulgaris

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SUMMARY

Propionibacterium acnes is the bacterial species most consistently isolated from acne lesions. Intradermal injection of a heat-killed suspension of *P. acnes* induced a delayed erythematous and often papular inflammatory reaction which was maximal after 24–48 h. This response was dose related and was probably mediated at least partly by immune mechanisms. In eighty-one subjects with acne of varying severity the intensity of the 48 h skin test reaction was significantly related to the severity of the acne. These findings indicate that the host response to *P. acnes* is an important variable in determining the severity of inflammatory acne.

Increased sebum excretion correlates closely with the severity of acne (Cunliffe & Shuster, 1969). There is abundant evidence that sebaceous gland activity is hormonally controlled (Pochi & Strauss, 1974). Nevertheless, neither hormonal influences nor a raised sebum excretion rate satisfactorily account for the inflammatory changes which characterize acne. *Propionibacterium acnes* regularly colonizes the depths of sebaceous follicles in great numbers and circumstantial evidence suggests a role for *P. acnes* in the induction of inflammation, but experimental evidence (Puhvel & Sakamoto, 1977) does not support the hypothesis (Strauss & Pochi, 1965) that inflammation is initiated by irritant fatty acids derived from sebum lipids by the action of bacterial lipases. By examining inflammatory skin test reactions to intradermally injected suspensions of killed *P. acnes* we have explored the possibility that an altered host response to *P. acnes* might be involved in the pathogenesis of the inflammation.

MATERIALS AND METHODS

Preparation of bacterial suspensions

P. acnes (NCTC 737) was incubated at 37°C for 48 h in Robertson's cooked meat broth (Oxoid). One millilitre of this culture was inoculated into 400 ml of Lemco broth and incubated for 5 d at 37°C as a

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static culture. The viable bacterial count was determined by the method of Miles and Misra (Miles & Misra, 1938). The organisms were separated by centrifugation, washed in saline and heated at 100°C in a water bath for 30 min. The heat-killed bacteria were resuspended at a concentration of 150×10^6 organisms/ml in Coca's solution (0.085 M NaCl, 0·03 M $NaHCO_3$ and 0·043 M phenol) and stored at 4°C. Further dilutions containing $0·15 \times 10^6$, $1·5 \times 10^6$ and 15×10^6 organisms ml in Coca's solution were prepared for intradermal injection. Sterility of the final bacterial suspensions was confirmed by aerobic and anaerobic culture.

Skin tests

The bacterial suspensions (50 μl) were injected intradermally into the flexor aspect of the forearms with a 26 gauge needle. All tests were performed in duplicate and a control injection of Coca's solution was included. The skin thickness was measured at all injection sites with Harpenden skin-fold calipers (Down Bros., London) before injection. The inflammatory response was recorded at 6, 24 and 48 h after injection and three parameters were assessed: (1) the diameter of erythema (mm); (2) the presence or absence of a papule and (3) the change in skin thickness (mm). The mean of duplicate results at each concentration was recorded for each patient.

Assessment of acne severity

Severity of acne was assessed by means of a cumulative points system before skin testing. One point was scored for the presence of each of the following: comedones, papules, pustules and cysts. Scarring was assessed as none (0 points), minimal (1 point), moderate (2 points) and severe (3 points). The presence of lesions on the face, back or chest scored 1 point for each area. The maximum score for the most severely affected patients was 10 points. The patients were then divided into 3 grades: grade 1—normal or minimal acne (0–3 points), grade 2—moderate acne (4–6 points), grade 3—severe acne (7–10 points).

Patients

Eighty-one subjects were investigated including seven with no evidence of acne. There were forty-five

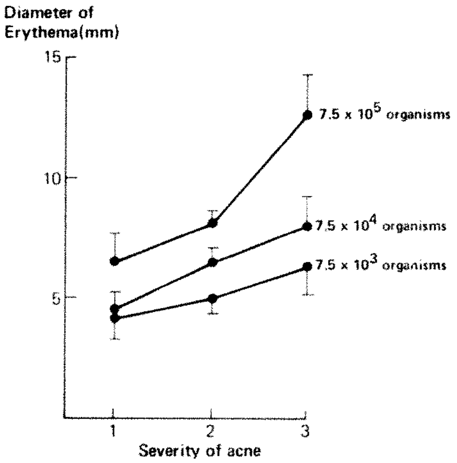

FIGURE 1. Erythema response at 48 h (±SE) related to severity of acne following injection of three different doses of *P. acnes*. $P < 0·01$, analysis of variance.

males and thirty-six females with a mean age of 21·8 years (range 13–37). Males comprised 43% of grade 1, 48% of grade 2 and 66% of grade 3 subjects. The range and mean age in each group were similar. Treatment was unaltered during investigation.

RESULTS

At control injection sites erythema did not exceed 2 mm and no papules developed. Compared with the control site, the sites of bacterial injection started to develop erythema after 6 h, reaching a maximum at 24–48 h and declined slowly thereafter. Skin thickness was frequently increased after 24–48 h by which time many reactions were papular. There were no significant differences between the 24 and 48 h measurements and only the 48 h results are therefore reported in this paper.

The erythema response, the proportion of papular responses and the measured increases in skin thickness were all dependent on the concentration of bacteria injected. At each of the three bacterial concentrations these parameters were all significantly related to the severity of acne (Figs 1–3).

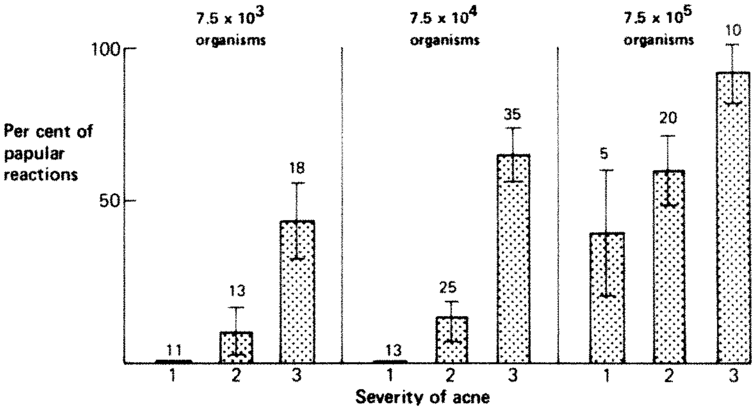

FIGURE 2. Proportion of papular reactions at 48 h related to severity of acne following injection of three different doses of *P. acnes*. Numbers in each group are indicated above bars. $\chi^2 = 42{\cdot}5$, $P < 0{\cdot}00001$.

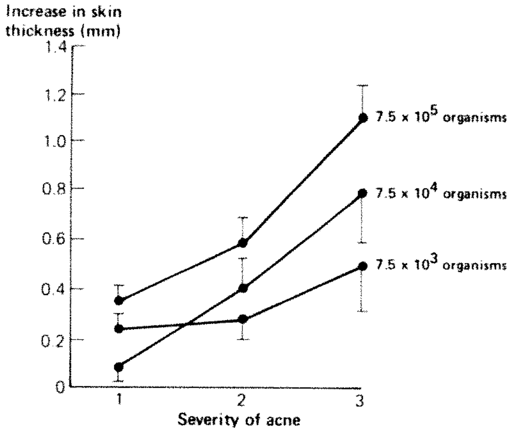

FIGURE 3. Increase in skin thickness at 48 h (±s.e. mean) related to severity of acne following injection of three different doses of *P. acnes*. $P > 0{\cdot}05$, analysis of variance.

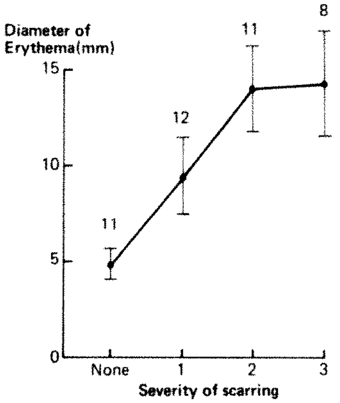

FIGURE 4. Erythema response at 48 h (±s.e. mean) related to severity of scarring following injection of $7{\cdot}5 \times 10^5$ organisms.

Disease severity was also assessed according to the degree of scarring, while ignoring active inflammation. The erythema responses were largest in individuals with moderate or severe scarring, intermediate in those with minimal scarring and smallest in those without scarring (Fig 4) but this trend was not statistically significant.

DISCUSSION

The results clearly demonstrate a direct relationship between the severity of acne and the host response to *P. acnes* as assessed by delayed skin test inflammation. A similar trend was reported in another study (Puhvel & Sakamoto, 1977), but the number of patients was small, no account was taken of different disease severity and only a single concentration of bacterial suspension (2×10^5 organisms) was used. Moreover erythema reactions were scored only as positive or negative by predetermined criteria thus limiting statistical evaluation.

The highest bacterial dose used in the present study resulted in the injection of $7{\cdot}5 \times 10^5$ organisms. Up to 10^7 viable *P. acnes* per follicle have been isolated from the back and frontal scalp of normal individuals (Puhvel, Reisner & Amirian, 1975) and the numbers are certainly higher in acne subjects. Our results therefore indicate that even killed *P. acnes* is capable of inducing significant inflammatory reactions in numbers which can be regarded as 'physiological' in the follicles of acne subjects.

The pathogenesis of the increased reactivity is at present unclear. Available evidence suggests the involvement of immune mechanisms. Complement (C_3) can regularly be observed in dermal vessel walls in early inflammatory acne lesions (Dahl & McGibbon, 1976; Scott, Cunliffe & Gowland, 1979; Dahl & McGibbon, 1979) and we have observed similar C_3 deposition in 24 h *P. acnes* skin test reactions (Kersey & Dahl, 1979). Complement fixing antibodies to *P. acnes* are found in high titre in the serum of patients with severe acne (Puhvel, Hoffman & Sternberg, 1966). In addition, direct activation of the alternate complement pathway by *P. acnes* and *Corynebacterium parvum* (now considered identical) (Cummins & Johnson, 1974) has been described (McBride *et al.*, 1975; Massey, Mowbray & Noble, 1978; Webster *et al.*, 1978). *In vitro* studies of lymphocyte transformation (Puhvel *et al.*, 1977) and macrophage migration inhibition (Gowland *et al.*, 1978) by *P. acnes* indicate enhanced cell-mediated hypersensitivity to the organism in severe inflammatory acne. It therefore seems likely that both humoral and cell-mediated hypersensitivity and perhaps non-antibody dependent complement activation contribute to the inflammatory skin test reactions that we have observed.

Delayed skin test reactions to various other viral, bacterial, yeast and fungal antigens have previously been reported to be reduced in patients with severe acne (Plewig & Kligman, 1975; Palatsi, 1977; Rajka, 1977). Moreover, anergy to DNCB was demonstrated in 4/4 patients with severe inflammatory acne (Palatsi, 1977). Thus the enhanced reactivity to *P. acnes* seems to be specific.

It is not clear at what stage of the disease increased reactivity to *P. acnes* first develops. Nevertheless, intact organisms are present in inflammatory lesions and our results suggest that the severity of inflammation is dependent not only on the absolute numbers of organisms but also on the individual host response that is elicited by the organism.

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Wheat grain immunofluorescent antibodies as an indication of gluten sensitivity?

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SUMMARY

An immunofluorescence method using whole sections of wheat grains as the substrate was applied to detect circulating antibodies to wheat gluten in dermatitis herpetiformis patients and in controls. Only IgG class antibodies were detected. From dermatitis herpetiformis patients 22% had these antibodies as had 22% of the atopic dermatitis group. Among the controls who had no skin problems 12% were faintly positive. It is evident that the test as such is non-specific and does not have diagnostic significance in dermatitis herpetiformis.

Dermatitis herpetiformis (DH) is an annoying itchy vesiculopapular skin eruption with a still unknown aetiology. Several factors indicate that autoimmune mechanisms are involved. However, the basic events are obscure (Katz & Strober, 1978). Deposits of immunoglobulin A (IgA) in the dermal papillae of uninvolved skin are considered highly pathognomonic for the disease (van der Meer, 1969; Seah & Fry, 1975).

Adult coeliac disease (ACD) is commonly associated with DH and, in both it is suspected that wheat related cereal proteins initiate the symptoms. In addition, in most DH patients an enteropathy like ACD can be found (Brow *et al.*, 1971, Reunala, 1978). Recently Eterman *et al.* (1977) described a simple immunofluorescent (IF) test to detect antibodies to wheat proteins in ACD patients using cryostat sections of wheat grains as a substrate for the test. In their series most of the patients were found to possess circulating IgG class antibodies to wheat proteins, most probably to gluten.

Wheat gluten and skin reticulin are found to show some cross reactivity (Seah *et al.*, 1972). Based on this finding an attempt has been made to explain the appearance of IgA aggregates in the upper dermis. However, further studies have not confirmed that reticulin may be the target in the skin (Stingl *et al.*, 1976).

The purpose of the present study was to investigate the possibility of detecting different antibody classes to wheat proteins, using the IF test described, in DH patients and in controls and, in addition, to test the cross reactivity of these antibodies to type III collagen, the most prominent component of reticulin, using the immunodiffusion technique. The specificity of the IF test is discussed.

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PATIENTS AND METHODS

Patients

The serum specimens were obtained from the Department of Dermatology, University of Turku, comprising the following: (a) fifty-five serum specimens from forty-six patients with DH (thirty male, sixteen female, age range 19–70 years); two or three specimens were obtained from seven patients with a minimum interval of 2 months; (b) fifty specimens from forty-five patients with atopic dermatitis (nineteen male, twenty-six female, age range 7–74 years); (c) three specimens from three females with lichen simplex chronicus (age range 40–61 years); (d) three specimens from three patients suffering from ACD (one male, two females, age range 21–42 years); (e) forty-two specimens from thirty-six patients with no history of skin or intestinal problems (sixteen males, twenty females, age range 19–70 years).

Of the serum specimens from DH patients, thirteen were obtained before any medication or diet was started; the rest of the specimens were obtained while the patients were advised to follow a gluten-free diet and/or were taking dapsone. (Part of this patient material was previously analysed for the presence of IgM class rheumatoid factor in the serum (Kalimo, 1978).

The serum specimens were stored at −20°C until used.

Serological tests

The IF test described by Eterman *et al.* (1977) was used. In brief, cryostat sections of whole wheat grain were incubated with serial dilutions of serum specimens. After incubation at 37°C for 30 min, the sections were washed and an aliquot of fluorescein labelled rabbit antihuman IgA, secretory piece IgA, IgG and IgM was applied. After an additional incubation and washing the sections were examined on a Leitz Orthoplan Fluorescence microscope. Optimal dilutions for each antiserum used were determined separately. A positive (sample from an ACD patient) and a negative control serum and a buffer blank were included in each test. The fluorescence pattern was graded as negative, faintly fluorescent (+) and heavily fluorescent (+ +).

The type III collagen (reticulin) was prepared as follows: growth media from confluent cultures of human diploid fibroblasts were changed to media without serum but supplemented with ascorbic acid (50 μg/ml). After 24 h the media were removed, dialysed against ice-cold distilled water and additionally against acetic acid. The collagen precursors were converted into collagen-like polypeptides by pepsin at 15°C; pepsin was inactivated at pH 8·0. Pepsin-treated type III collagen was then purified in 4 M urea using carboxymethylcellulose chromatography (Piez, Eigner & Lewis, 1963) and lyophilized to be used in immunodiffusion.

Three patients with atopic dermatitis whose sera gave a positive result in the IF test were further studied with skin prick testing with wheat, barley, oats and rye flour. In the skin test a weal 50% or larger in diameter than that of histamine 1:1000 was evaluated as positive (+ +). The serum specimens of these three patients were further analysed with an IgE radioallergosorbent test (RAST) for the presence of cereal specific IgE antibodies (Hoffman, 1975). A careful history of the effect of cereals was recorded.

RESULTS

The structural components of wheat grains were easy to recognize in IF. The outer, cuticulous layer showed a heavy autofluorescence. In the inner part of the grain, both starch granules and a lacy protein network containing gluten were clearly visible with no autofluorescent pattern.

In the patient sera only IgG class IF antibodies to the wheat grain protein could be detected. The localization of the fluorescence corresponded to that of gluten. The positive control specimen showed

TABLE 1. Immunoglobulin G immunofluorescent antibodies to wheat grain section in different patient groups

	IgG++		IgG+		IgG−			
Clinical diagnosis	No. of specimens	%	No. of specimens	%	No. of specimens	%	No. of specimens	No. of patients
Dermatitis herpetiformis	12	22	19	35	24	43	55	46
Atopic dermatitis	11	22	9	18	30	60	50	45
Lichen simplex chronicus	1	—	—	—	2	—	3	3
Adult coeliac disease	1	—	—	—	2	—	3	3
No skin problems	—	—	6	14	36	86	42	36

TABLE 2. Results of skin prick testing in three patients demonstrating circulating immunoglobulin G antibodies to wheat grain sections in immunofluorescence test

	Prick testing			
	Wheat	Oats	Barley	Rye
Patient 1	++	−	−	−
Patient 2	−	++	++	−
Patient 3	−	−	−	−

a granular fluorescence whilst the other samples showed a more diffuse pattern. The localization was, however, similar.

The number of positive specimens in each patient group is indicated in Table 1. In DH patients IgG antibodies were detected in twelve out of the fifty-five specimens tested. In addition, a faint positive reaction was seen in nineteen specimens. No significant correlation was found with the severity or duration of the disease, diet or medication and the detection of circulating antibodies. In patients with atopic dermatitis a positive fluorescence was seen in eleven out of the fifty specimens and a faint positive one in nine out of fifty. One of the patients with lichen simplex chronicus had IgG IF antibodies. In the patients with no skin problems six showed a faint fluorescence indicating possibly the presence of IgG antibodies. One of the ACD patients had IgG IF antibodies while the other two remained negative.

In immunodiffusion against the pepsin-treated type III collagen none of these specimens showed any precipitation lines. Only the specimens of DH patients were tested.

The results of prick testing in three patients with atopic dermatitis are shown in Table 2. Patient 1, who had an allergic reaction to wheat, also had symptoms (urticaria) when eating cereal products. The other two patients could not discover any correlation between their symptoms and the handling or eating of cereals. Patient 2 had, however, a positive reaction to oats and barley in the prick test. By RAST no specific IgE antibodies against wheat, barley, oats or rye in the serum of these three patients could be detected. Their total IgE was also within normal levels.

DISCUSSION

It is evident that the IF test described is a simple method of demonstrating antibodies to wheat grain structures, but the specificity of the test remains questionable. Out of the fifty-five specimens from DH patients which were tested, 22% were positive demonstrating IgG class antibodies, and furthermore 35% were faintly positive. However, in our series, a relatively high percentage of positive specimens was found in other patient groups, too. Altogether 22% of the patients with atopic dermatitis showed a significant pattern and, in addition, 18% were faintly positive. In the control group where no skin problems were present, 12% were faintly positive. This proves that the test as such does not have diagnostic significance in DH.

Allergic reactions to the food proteins may be mediated by different antibody classes (May & Bock, 1978; Parish, 1970). In that respect three atopic patients were also skin prick tested and furthermore circulating specific IgE antibodies were determined. In one case a positive correlation with the clinical history and the IF and prick test results was achieved. The negative RAST result may simply reflect the fact that the patient had been avoiding cereals for a year and the level of circulating IgE antibodies had become undetectable. The other two patients had no evidence of cereal intolerance. Because the skin prick tests are prone to false reactions, the positive result for the other may have been nonspecific.

Before any conclusions about the possible cereal sensitivity can be drawn more extensive studies will be required with larger numbers of patients and with more specific and sensitive tests.

In immunodiffusion none of the samples showed antibodies to the purified type III collagen. Even though it has been suggested that gluten and reticulin have common antigenic determinants (Seah *et al.*, 1972), the localization of IgA in the dermal papillae has not appeared to correspond to that of reticulin. Our negative immunodiffusion result parallels the findings that reticulin is not the target in the skin. On the other hand, in pepsin treated type III collagen preparations the telopeptide regions are cleaved. It is thus possible that the antigenic determinants responsible for cross-reactions in the gluten sensitivity were not present in the preparations.

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Pharmacology and Treatment

Treatment of dermatitis herpetiformis with cholestyramine

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SUMMARY

Two patients with clinically and histologically typical dermatitis herpetiformis showed a dramatic clearing of their skin lesions in association with an oral dose of 4 g of cholestyramine three times a day. Reappearance of the lesions occurred on the discontinuance of the therapy, followed by remission on resumption of cholestyramine.

It is postulated that the non-absorbable anion exchange resin, cholestyramine, may have bound and hence blocked absorption of an unidentified compound (possibly gluten, iodine, or endotoxin) critical to the pathogenesis of dermatitis herpetiformis.

A linkage between the bowel and skin disease has never been more clearly revealed than in dermatitis herpetiformis (Alexander, 1975), since these patients almost invariably show atrophic inflammatory changes in the small bowel (Thune *et al.*, 1979). Significantly, the bowel changes have been traced to a sensitivity to the cereal protein, gluten, in the diet (Cooper *et al.*, 1978). Elimination of this gluten for a period of months leads to return of normal mucosal histology and a variable degree of lessening of the skin lesions (Reunala *et al.*, 1977). Thus, gluten sensitivity may play a role in the pathogenesis of dermatitis herpetiformis, but the picture is puzzling because a gluten-free diet does not always clear the skin, and skin tests to gluten are negative (Marks, 1977).

The present observations were made to explore further the possibility that the skin lesions of dermatitis herpetiformis might be the result of absorption of an antigen or toxin from a gluten damaged small bowel mucosa. Cholestyramine resin was prescribed to reduce or block the absorption of such compounds. This resin is the insoluble chloride salt of a non-absorbable styrene divinylbenzene copolymer. As a strongly basic exchange resin, it binds not only bile salts, but sequesters many acidic drugs and other compounds (antibiotics, aspirin, barbiturates, cyanocobalamin, digitalis, iodine, iron, phenylbutazone, thiazide, thyroid, vitamin A, D, E) as well as endotoxins (West & Lloyd, 1975; Nolan & Ali, 1972; Ti, Giles & Sellers, 1978).

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CASE REPORTS

Case 1

This 68-year-old white female has had a severely pruritic, widespread, vesiculobullous eruption which clinically and histologically has been diagnosed as dermatitis herpetiformis since 1976. Hospitalization at that time revealed no systemic disease on extensive blood studies, both haematological and chemical, nor on radiographs of the chest, entire bowel and renal system. Control of secondary infection was achieved with systemic ampicillin and erythromycin.

The disease has continued to the present time despite reduction of gluten and iodine intake, and elimination of sea food from the diet. Intramuscular steroid injections, topical steroid creams, as well as hydroxyzine and cimetidine provided some relief. Dapsone was not given because of a questionable history of sulphone sensitivity.

Complete involution of the vesiculation, however, was achieved only by oral administration of cholestyramine resin in a dose of 4 g three times daily. This was given from August 1978 to February 1979. Following re-exacerbation of the disease, a second course was given from September 1979 to November 1979, with complete suppression of the lesions but not of the pruritus. Currently she has been taking a third course of cholestyramine for the past month, again with complete disappearance of all the vesiculobullous lesions which appeared 6 weeks ago. She has tolerated the drug well.

Case 2

This 61-year-old white male has had a severely pruritic, vesicular, pigmented eruption of the buttocks, thighs, arms and scalp for over 11 years. The diagnosis of dermatitis herpetiformis was made 10 years ago on clinical grounds and on the histological findings in two biopsies. Treatment had centered on systemic steroids, a gluten free diet, and dapsone. The steroids had to be discontinued because of side-effects, the gluten diet for one year had no significant effect, and after 4 years of help with dapsone he developed an agranulocytosis requiring white cell transfusions. At that time he was hospitalized and a jejunal biopsy revealed inflammatory changes. Recently, administration of oral cholestyramine (4 g three times daily) was followed within one week by a remarkable involution of his widespread vesicular lesions. He reported that his skin felt 'the best since childhood'. The cholestyramine has been continued for the past 2 months with no new lesions appearing. Topical treatment has been limited to the steroid cream used prior to the cholestyramine.

DISCUSSION

The concept that pathogenic materials are being absorbed from a damaged gut in patients with dermadermatitis herpetiformis is an attractive one (Davies, Marks & Nuki, 1978; Hemmings, 1978). The studies reported here grew out of an attempt to block the absorption of such a postulated agent by having it bind to a non-absorbable resin, cholestyramine. Classically this resin has been used in the control of hypercholesterolaemia, by virtue of its bile acid sequestrant action and consequent interference with the enterohepatic bile salt cycle. Later, it became popular in the treatment of pruritus associated with liver disease such as biliary cirrhosis or cholestasis (Laatikainen, 1978), polycythaemia vera (Chanarin & Szur, 1975) and uraemia (Silverberg *et al.*, 1977). It has more recently received wide use in the treatment of diarrhoeal states induced by bile acids (Duncombe, Balin ; Davis, 1977; Heusinkveld, Manning & Aristizabal, 1978), as well as endotoxins (Kreutzer & Milligan, 1978).

Cholestyramine is remarkably free of side-effects since it is not absorbed from the bowel. Some patients experience constipation or, with very high doses, diarrhoea, heartburn and nausea. However, the impairment of absorption of drugs, fat soluble vitamins, folates, and possibly calcium is the key adverse phenomenon in a drug that may be taken safely over a period of years (Wade, 1977). Despite its apparent inertness, cholestyramine has been shown to alter enzyme activity in isolated ileal cells from rats (Panini *et al.*, 1979) and possibly to have a role in inducing intestinal tumours (Sachatello, 1979).

In the present study, cholestyramine has proven to be a new form of therapy for dermatitis herpetiformis. The mechanism of action remains unknown but of especial interest is the fact that the absorption of both iodine and bacterial endotoxins is reduced by cholestyramine (Nelson, 1974; Nolan & Ali, 1972). Iodine has long been known not only to cause exacerbations of dermatitis herpetiformis, but actually to initiate the disease (From & Thomsen, 1974; Douglas & Alexander, 1975). It is possible that cholestyramine may act by reducing the absorption of iodine to below critical levels. Its role in reducing endotoxin absorption is also intriguing because in dermatitis herpetiformis there is an overgrowth of bacteria in the small bowel (Heading *et al.*, 1974), possibly due to the atrophic gastritis which may develop. Cholestyramine may also be binding critical acidic fractions of gluten and its component gliadins and glutenins.

The use of cholestyramine as an inert non-absorbable agent to bind pathogenic agents in the gut of dermatitis herpetiformis patients is a new concept. Inasmuch as dietary fibres, such as bran or sunflower seed, also have cholestyramine-like binding powers (Oakenfull & Fenwick, 1978), the observations reported also have implications for further study of the role of diet itself. It would appear that both anion and cation exchange resins as well as 'high fibre' diets deserve further study not only in dermatitis herpetiformis, but also in other diseases where there may be a direct relationship between what is absorbed from the gut and what is happening in the skin.

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Cryosurgery in the treatment of lichen sclerosus et atrophicus of the vulva

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SUMMARY

Twelve patients with lichen sclerosus et atrophicus of the vulva were treated by a nitrous oxide cryoprobe. All had had severe itching which was relieved in the post-operative period in 75% and for 3 years in 50%. Eight had histologically proven leukoplakia which cleared post-operatively in five (62·5%) and remained clear at three years in four (50%). In one patient pruritus, leukoplakia and ultimately squamous carcinoma appeared.

The majority of patients with lichen sclerosus et atrophicus of the vulva (L.S.A.V.) are troubled by pruritus, soreness, dysuria and dyspareunia; only a few are asymptomatic (Wallace, 1971). Many therapies have been recommended. Topical steroids are the most frequently used treatment, but later on in the condition these agents may be ineffective. Topical oestrogen is useful in children (Lascano, Montes & Maggini, 1964). Denervation of the vulva by alcohol injection helped a high proportion of patients (Ward & Sutherst, 1975). Excision of the vulva is usually followed by a return of pruritus, and radiotherapy could precipitate malignancy (Hart, Norris & Helwig, 1975). Leukoplakia develops in as many as 50% of patients and has been regarded as an indication for local excision or vulvectomy, even though the grafted skin may again develop both lichen sclerosus et atrophicus and leukoplakia (Whimster, 1973). L.S.A.V. can therefore be most difficult to treat. A cryosurgical method of treatment that would destroy leukoplakia, relieve pruritus and which could be repeated if necessary seemed to offer advantages.

MATERIAL

We investigated and treated twelve women who were attending the Skin Out Patients Department with lichen sclerosus et atrophicus of the vulva complicated by leukoplakia judged to be present on clinical grounds. Our criteria are a thickened white plaque with intense itching, fissuring or persistent ulceration. We consider leukoplakia to be present histologically when there is thickening of the

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stratum corneum and epidermis with groups of denticulate processes budding down from the basal layer. In the majority, dysplasia of keratinocytes is also present, but occasionally the dysplasia is seen only when the denticulate processes have become overtly carcinomatous (Wallace, 1971). Histologically leukoplakia was found to be present in eight patients and absent in four. The condition had escaped control by topical corticosteroids so that medical management was deemed to have failed. Six patients had previous surgical vulvectomies and two had had radiotherapy. The age range was 46–74 years with a mean of 63·3. The duration of the disease varied from 3 to 35 years with a mean of 16·5.

METHOD

All the patients were assessed clinically and histologically by biopsy under general anaesthesia. The biopsied sites were carefully charted. The whole vulva was frozen under general anaesthesia as it was desired to relieve pruritus in the entire area. An Amoil TCC cryo unit (Spembley Ltd) was used. This machine has a metal probe with an elliptical end 1·5 × 1·2 cm, which is placed in contact with the skin and then cooled to −80°C by nitrous oxide. The probe was applied for 1 min, area by area until the whole vulva had been treated. Blistering usually developed the following day, but with relatively little discomfort. After 3–4 days, daily baths were encouraged and applications of astringents were made to the vulva. The patient left hospital when she felt able to manage her dressings at home.

When the vulva was fully healed the patient was submitted to further biopsies after a minimum interval of 10 weeks. Because of difficulties in obtaining tissue from areas exactly comparable with the original biopsy, it was our policy to take an assessment biopsy from the most severely affected area. It seemed logical to seek information about the pathological status of the worst looking area of the vulva. Four patients had a repeat application of the cryoprobe and a further patient required two repeat applications.

Follow up data were obtained after 3 years from the clinician in charge of the patient.

RESULTS

The immediate effect of the freezing was to produce severe blistering. Although the time of application of the cryoprobe was constant, one patient sustained an area of full thickness skin loss, while in others

TABLE 1. Summary of results in twelve patients with lichen sclerosus et atrophicus treated with cryoprobe

	Immediate (No. of patients)		After 3 years (No. of patients)	
Excellent: no further therapy required	3	(25%)	3	(25%)
Excellent: further treatment with topical steroids required	6	(50%)	2*	(16%)
Failure	3	(25%)	4	(33%)
Disastrous progression	—		1	(9%)
No follow up data	0		2	(18%)

* One of these two patients relapsed after 2½ years but improved after further cryotherapy.

TABLE 2. Results of treatment of leukoplakia presenting originally in eight patients

	No. of patients	
Clear after initial therapy	3	(37%)
Early relapse, cleared after repeat treatment	2*	(25%)
Clear after 3 years	4	(50%)
Late relapse	1	(12·5%)
Unchanged	1	(12·5%)
Squamous cell carcinoma	1	(12·5%)

* See Table 1.

the loss was partial. Healing times, therefore, varied from 2 weeks to 3 months. The results are given fully in Tables 1 and 2; assessments were made between 10 and 20 weeks after treatment. Pruritus was relieved with or without the use of topical steroids in nine patients and only three were no better. After 3 years five patients remained satisfactory, five suffered an early relapse or had no relief at all and two were lost to follow-up.

Leukoplakia was suspected on clinical grounds in all twelve patients but was confirmed histologically in only eight; three were clear after initial therapy and a further two cleared after the therapy was repeated. One patient relapsed after 2½ years and another patient was unchanged; one patient, who had a radium implant 15 years ago, progressed disastrously to squamous cell carcinoma. She had leukoplakia prior to treatment but histological examination showed this to be clear after cryotherapy. It would appear that radiation had added to the malignant potential of the lichen sclerosus. After 3 years, half of our patients remained clear of leukoplakia.

COMMENT

We believe that useful results were obtained in this study. Half of our patients were relieved of pruritus and leukoplakia for 3 years when the other therapeutic options were to endure the condition or to have a vulvectomy. The operation of vulvectomy requires considerable skill, a prolonged stay in hospital and is attended by a significant incidence of vaginal stenosis. We suggest that cryosurgery might have some advantages in this context. Freezing is seldom followed by haemorrhage because of spontaneous vascular stasis. Unlike radiotherapy and cautery, cryosurgery causes little scarring and can be repeated without the risk of vaginal stenosis; although healing of lesions takes up to 6 weeks, pain is minimal. This may be because the arrangement of collagen bundles in the dermis is not disturbed as these fibres are relatively resistent to cold damage. Presumably new collagen is orientated in the same way as the old so that dense scar collagen is not formed. Pruritus may be relieved because of damage to cutaneous nerves which certainly occurs in the successful alcohol injection technique (Ward & Sutherst 1975). There may, however, be other explanations for this phenomenon. Various methods of cryosurgery are available. Scott (1975) has developed a technique using liquid oxygen and nitrogen applied on cotton wool swabs, but apparatus which sprays liquid nitrogen is more convenient. Liquid nitrogen has the advantage of producing rapid widespread cooling, but in practice it can be difficult to prevent freezing of the clitoral area. This produces swelling of the urethral opening and retention of urine. The cryoprobe method takes more time and it can be difficult to prevent overlap of treated areas. The same would apply to techniques using cotton wool swabs.

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Oxatomide: *in vitro* assessment of antagonistic activity, and effects on histamine release and enzymatic histamine degradation in skin

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SUMMARY

The antagonistic activity of oxatomide, and its effects on evoked histamine release and histamine-N-methyl transferase activity in skin, have been studied. Oxatomide antagonizes H1 activity in a dose-dependent but non-competitive manner. It also shows some atropine-like activity. Oxatomide did not cause detectable inhibition of antigen-stimulated histamine release from skin slices of sensitized guinea-pigs although the possibility that oxatomide may cause weak inhibition could not be excluded. In the presence of low concentrations of histamine, oxatomide suppressed human skin histamine-N-methyl transferase, but in the presence of higher substrate concentrations it enhanced activity of this enzyme. These observations, which were limited by the poor solubility of oxatomide in aqueous media, should encourage further *in vivo* studies of oxatomide's histamine-suppressing properties in the human subject.

Conventional (H1) antihistamines, although widely prescribed, are often disappointing in clinical practice. Attention has recently turned to drugs which suppress histamine release from mast cells, including disodium cromoglycate (Cox, 1967). This drug is effective in allergic respiratory and bowel disease but is inactive systemically, and is of little value in the skin. Oxatomide, which has the structure 1-{3-[4-(diphenylmethyl)-1-piperazinyl]propyl}-1, 3-dihydro-2H-benzimidazol-2-one, is a novel compound which is said to possess properties of both histamine antagonism and suppression of histamine release from mast cells (Awouters *et al.*, 1977). It is of special interest dermatologically that these workers demonstrated inhibition of both passive cutaneous anaphylaxis and reactions to intradermal histamine injection in the rat by orally administered oxatomide. As a preliminary to possible studies of this drug in human subjects, we have investigated the antagonistic activity of oxatomide on the histamine and acetylcholine receptors of the guinea-pig ileum. We have also evaluated the effect of oxatomide on spontaneous and antigen-evoked histamine release from guinea-pig skin *in vitro*, and on enzymatic catabolism of histamine by human skin histamine-N-methyl transferase.

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MATERIALS AND METHODS

Solubility of oxatomide

Oxatomide was found to be poorly soluble in aqueous media, especially when phosphate buffer was present in solution. Accordingly, organic solvents had to be mixed with water to dissolve the oxatomide. Interference of organic solvents with the responses of the tissue under study limited the proportion of organic solvent which could be added, which in turn limited the highest concentration of oxatomide which could be studied.

Antagonistic activity

Preparations of longitudinal muscle with adhering myenteric plexus were prepared from the guinea-pig ileum, and suspended under 0·4 g tension in a 3 ml organ bath containing Krebs solution at 37°C, and bubbled with 95% oxygen/5% carbon dioxide. The contractile responses of the muscle were recorded isometrically and displayed on an ink-writing pen oscillograph. The dissociation equilibrium constant (K_e) was determined by the method of Arunlakshana & Schild (1959), $pA2$ being equal to $-\log K_e$. For competitive antagonists dose-response curves in the presence of increasing concentrations of antagonist are parallel to the control dose-response curve (no antagonist) but are displaced to the right. When the concentration of histamine which gives 50% of the maximal response (ED50) in the absence of antagonist is designated a, and the concentration of histamine giving the same response in the presence of the antagonist is A, then the dose ratio, $DR = A/a$. Plots of log $(DR-1)$ against log antagonist give a straight line with a slope of unity which intersects at the abscissa at $DR-1 = 1$ to give the $pA2$. For non-competitive antagonists, the dose response curves in the presence of the antagonist are not parallel to the control curve and do not reach the same maximum response; therefore the relationship of log $DR-1$ against log antagonist is not applicable.

Preliminary experiments showed that dimethyl formamide (DMF) in final bath concentrations of up to 0·3% did not affect the assay tissue, although higher doses did affect the response to histamine and to electrical field stimulation at 0·1 HZ (Cowie, Kosterlitz & Waterfield, 1978). To overcome the poor solubility of oxatomide in water, oxatomide was dissolved in a 1·5% solution of DMF in a modified Krebs solution. The maximum solubility of oxatomide in this vehicle was 36 μg/ml and the stock solution used in these experiments was 25 μg/ml. When aliquots of 20–200 μl of this were added to the organ bath, the final concentration of DMF was 0·01–0·1% and the maximum molarity of oxatomide which we could investigate was $5{\cdot}4 \times 10^{-6}$M.

Histamine release

Male guinea-pigs weighing 400–500 g were injected subcutaneously in each hind limb with 0·5 ml of 1% ovalbumin (Sigma) in 0·9% sodium chloride solution. Three weeks after injection the animals were killed by stunning and exsanguination, and the abdominal skin was shaved and separated from subcutaneous tissues. Slices of approximately 500 μm thickness were obtained with a hand microtome as described by Yeoh, Tay & Greaves (1972). Samples were prepared containing five to six slices each in Tyrode solution. Each experiment was done in triplicate. Samples were preincubated for 15 min with fresh Tyrode solution with or without oxatomide 1–100 μg/ml in 0·1–0·4% DMF or 1% ethanol. At 100 μg/ml in 1% ethanol oxatomide remained partly in suspension, and the concentration of oxatomide in this solution is not known. The samples were then incubated for 5 min at 37°C in the presence or absence of ovalbumin antigen (10^{-4}g/ml) in a total volume of 4 ml. All samples contained aminoguanidine (10^{-5}M) to prevent degradation of histamine released during incubation (Yamamoto, Francis & Greaves, 1976). After incubation, the supernatant was removed for assay and replaced with 5 ml Tyrode solution, and heated in a boiling water bath for 5 min to extract residual histamine. Estimation of released and residual histamine was by fluorometric assay, using O-phthalaldehyde (Sigma Chemical Ltd) as a fluorophore and a Technicon AA II autoanalyser as previously described (Eady *et al.*, 1979). Histamine release in each sample was expressed as a percentage of the total histamine present in the sample using the following formula:

$$\%\ \text{release} = \frac{A}{A+B} \times 100$$

where A is the histamine in the supernatant solution after incubation and B is the residual histamine. In expressing results, all antigen-induced histamine releases were corrected by subtracting the corresponding spontaneous release.

Histamine-N-methyl transferase

Histamine methyl transferase (HMT) activity was assayed by a modification of the method of Snyder & Axelrod (1965) described in detail elsewhere (Francis, Thompson & Greaves, 1980). Human skin, obtained from plastic surgery, was stretched, and the epidermis and upper dermis removed by scraping. The scrapings were homogenized and the homogenate centrifuged. The enzyme-rich supernatant was freeze-dried after dialysis, and used as a source of HMT. In order to determine the influence of oxatomide on HMT activity the enzyme was dissolved in 0·05 M phosphate buffer (pH 7·4). Incubations were carried out in triplicate for 30 min at 37°C. The total incubation volume of 500 μl contained the enzyme, ^{14}C-methyl S-adenosyl methionine at 4 μCi/μmol (final concentration 50 μM), histamine in concentration 0–100 μM and 0·05 M phosphate buffer (pH 7·4) with or without oxatomide. The low solubility of oxatomide in aqueous phosphate buffer made it necessary to dissolve the drug in absolute ethanol, and add an appropriate volume of this solution to make up the required concentration of oxatomide in the phosphate buffer. Under these conditions the concentration of oxatomide which could be studied (7·3 μg/ml) was limited by the effect of ethanol on HMT activity.

RESULTS

Histamine antagonism

In five experiments in which the sensitivity of the guinea-pig ileum preparations to histamine was normal (ED50 = $3{\cdot}155 \pm 1{\cdot}39 \times 10^{-6}$M (mean ± s.e.mean)) oxatomide, in concentrations of less than 2×10^{-8}M, was without effect on the responses to histamine. In the dose range 2×10^{-8}M–$3{\cdot}2 \times 10^{-7}$M, oxatomide antagonized histamine in a dose-dependent but non-competitive manner, the slope of the dose-response curve becoming progressively flatter as the concentration of oxatomide was increased until at $3{\cdot}2 \times 10^{-7}$M the curve was almost flat (Fig. 1). Although it is not possible to calculate a K_e for a non-competitive antagonist, the results indicate that a dose-ratio of 2 would be achieved with an oxatomide concentration of approximately 2×10^{-8}M. Over the dose range 2×10^{-8}M–$3{\cdot}2 \times 10^{-7}$M, oxatomide does not affect the responses of the tissue to added acetylcholine indicating that this action is specific for H1 receptors and not a non-specific toxic effect on the smooth muscle.

Acetylcholine antagonism

The mean ED50 for acetylcholine in five preparations was $1{\cdot}84 \pm 0{\cdot}52 \times 10^{-7}$M (mean ± s.e.mean). Oxatomide, at concentrations of up to 2×10^{-7}M, did not affect the dose response curve for acetylcholine. At doses of oxatomide between 3×10^{-7} and $5{\cdot}4 \times 10^{-6}$M, the responses to acetylcholine were depressed in a dose-related manner, the dose-response curves being shifted progressively to

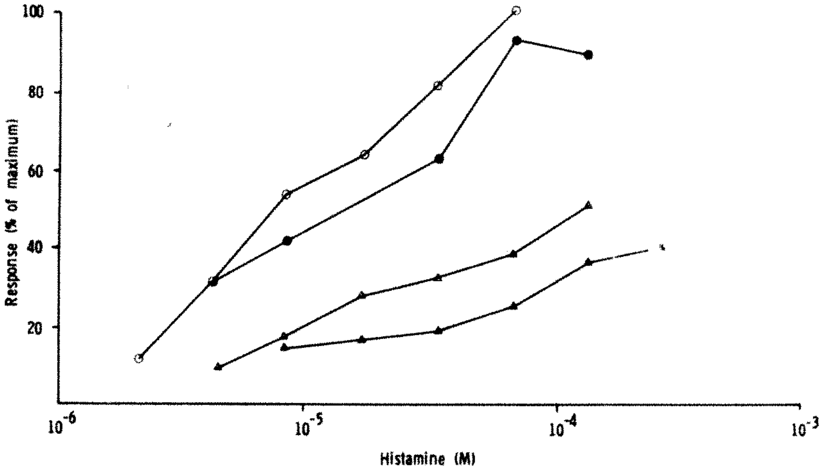

FIGURE 1. Antagonism of histamine response by oxatomide. The graph shows a single typical experiment. Ordinate = contractile response of guinea-pig isolated ileum preparation expressed as % of maximum. Abscissa = final molar concentration of histamine in organ bath. ○ = no oxatomide (control); ● = 2×10^{-8}M oxatomide; △ = 8×10^{-8}M oxatomide; ▲ = $1{\cdot}6 \times 10^{-7}$M oxatomide.

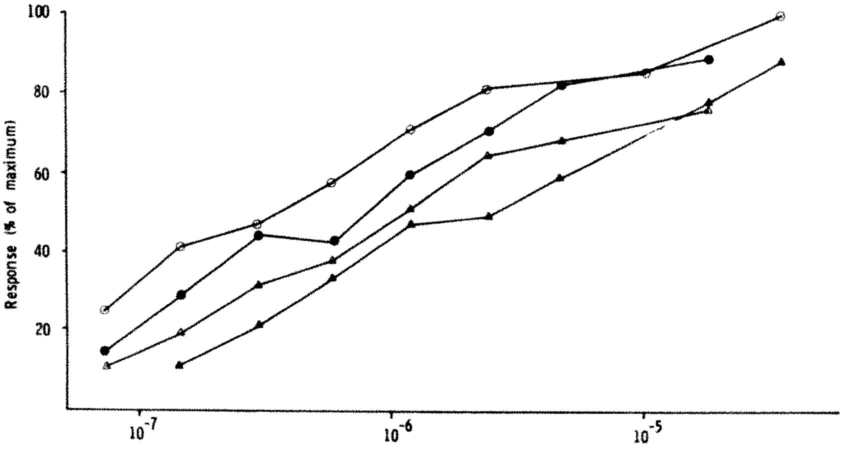

FIGURE 2. Antagonism of acetylcholine receptors by oxatomide. The graph shows a single typical experiment. Ordinate = contractile response of guinea-pig isolated ileum preparation expressed as % of maximum. Abscissa = final molar concentration of acetylcholine in the organ bath. ○ = no oxatomide (control); ● = $1{\cdot}35 \times 10^{-6}$M oxatomide; △ = $2{\cdot}7 \times 10^{-6}$M oxatomide; ▲ = $5{\cdot}4 \times 10^{-6}$M oxatomide.

the right with increasing doses of oxatomide (Fig. 2). However, it was not possible to show conclusively whether this antagonism was competitive or not, since the limited solubility of oxatomide restricted the concentrations to those producing a dose ratio of 12·5 or less. In these experiments the slope of the curve appeared to be reduced with the higher concentrations. The slope of log DR–1 against log oxatomide concentration was 1·418±0·182 ($n = 3$) which is steeper than that expected from a competitive antagonist. In four experiments, the dose of oxatomide required to give a dose ratio of 2 with acetylcholine was $7{\cdot}407 \pm 3{\cdot}413 \times 10^{-7}$M.

Histamine release

The results of four experiments are summarized in Table 1. In three of the four experiments it was found that the solvent alone in the concentration used (ethanol 1%; DMF 0·1–0·4%) caused inhibition of antigen-stimulated histamine release, ranging from 15·3 to 43·5% of the control value. These concentrations of solvents had no consistent effect on 'spontaneous' histamine release for skin samples which had not been challenged by antigen, but which had otherwise been treated in a similar way. The presence in the incubation medium of oxatomide 1–16 μg/ml did not cause further inhibition; on the contrary in experiments I, II and IV oxatomide appeared to mitigate the inhibitory effects of the solvent. In experiment III both oxatomide and DMF caused amplification of histamine release. The precise concentration of oxatomide which was in solution in experiment I is uncertain, since although 100 μg/ml oxatomide was mixed with 1% ethanol, it failed to enter solution completely.

TABLE 1. Antigen-evoked histamine release: effect of oxatomide

Experiment	Control	Oxatomide + solvent	Solvent
I*(a)	41·4	31·9	23·4
(b)	0·6	1·7	5·3
(c)	—	22·9	43·5
II†(a)	14·7	11·2	12·5
(b)	6·4	1·9	3·9
(c)	—	23·7	15·3
III‡(a)	29·2	37·0	42·7
(b)	7·3	6·8	2·7
(c)	—	−26·7	−46·2
IV§(a)	29·2	29·8	23·8
(b)	7·3	5·9	12·2
(c)	—	−2·00	18·5

* Oxatomide 100 μg/ml as a suspension in 1% ethanol.
† Oxatomide 16 μg/ml in 0·4% DMF.
‡ Oxatomide 1 μg/ml in 0·1% DMF.
§ Oxatomide 10 μg/ml in 0·4% DMF.

Values in (a) and (b) represent histamine release as % of total (see materials and methods): (a) = antigen-stimulated histamine release after subtraction of corresponding negative control 'spontaneous' histamine release; (b) = negative control 'spontaneous' histamine release (no antigen); (c) = inhibition of antigen-stimulated histamine release expressed as % of control value (no inhibitor). All values of (a) and (b) are means of triplicate samples.

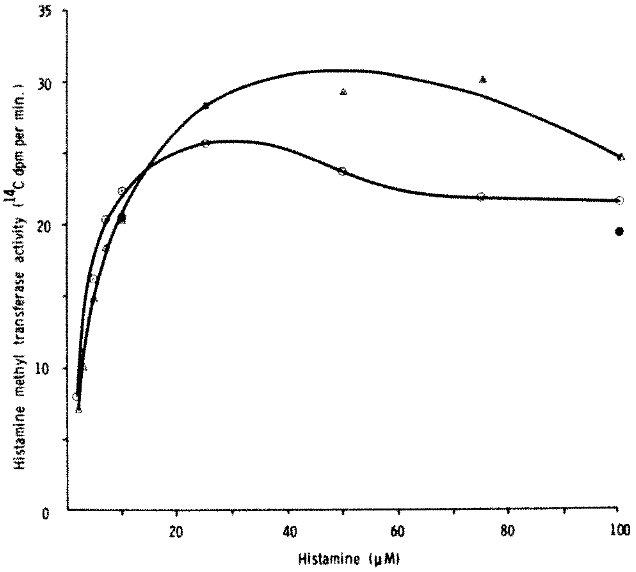

FIGURE 3. Effect of oxatomide on human skin histamine methyl transferase activity. Ordinate = enzyme activity as dpm of ^{14}C-methyl histamine formed and extracted per minute of incubation. Abscissa = histamine concentration (μM). ○ = mean enzyme activity in phosphate buffer containing 0·365% of ethanol. △ = mean enzyme activity obtained in the presence of oxatomide 7·3 μg/ml. ● = enzyme activity in absence of oxatomide and ethanol.

Histamine methyl transferase activity

The activity of HMT was measured over a histamine concentration range of 2–100 μM in phosphate buffer, containing 0·365% ethanol, with or without oxatomide at a concentration of 7·3 μg/ml. The results are shown in Fig. 3. HMT exhibits substrate inhibition at concentrations above 10 μM, resulting in a decrease in HMT activity at concentrations of histamine from 25 to 100 μM. At histamine concentrations of 25–100 μM, oxatomide partially relieves the substrate inhibition by histamine, resulting in an increase in HMT activity. At 10 μM and 100 μM concentrations of histamine, ethanol by itself appears to influence HMT activity.

DISCUSSION

The poor solubility of oxatomide in aqueous solvents limits the information which can be gained from *in vitro* studies. Nevertheless, oxatomide does exhibit fairly potent antihistamine activity which in concentrations of less than 2×10^{-7}M is specific for H1 receptors. However, its potency is only about 0·005 of that of mepyramine in producing a dose-ratio of 2 (Coutts & Greaves, 1980, in preparation). The nature of the antagonism appears to be non-competitive since addition of higher concentrations of histamine does not overcome the block due to oxatomide. Like most H1 antihistamines, oxatomide shows some anti-muscarinic activity, although only at concentrations considerably higher than those causing significant antagonism of histamine. A comparison of the anti-muscarinic activity of atropine (K_e $7{\cdot}60 \pm 1{\cdot}891 \times 10^{-10}$M, $n = 3$) with oxatomide ($7{\cdot}407 \pm 3{\cdot}413 \times 10^{-7}$M) suggest that atropine is about 1000 times more potent than oxatomide as an anti-muscarinic agent.

It has previously been stated that oxatomide inhibits histamine release due to systemic anaphylactoid reactions in the dog (Awouters *et al.*, 1977). Our *in vitro* results with antigen-evoked histamine release from guinea-pigs sensitized for anaphylaxis do not support this claim over a limited concentration range of oxatomide, although we are unable to exclude a small inhibitory action on stimulated histamine release since a modest suppressive effect could have been masked by the effect of organic solvents used, which themselves caused inhibition. It is of interest that an inhibitory action of ethanol on anaphylactic histamine release has previously been described (Schild & Mongar, 1957). We are unaware of any previous reports that DMF inhibits anaphylactic histamine release.

The effects of oxatomide on HMT activity in human skin were of special interest. Oxatomide had a dual effect on HMT activity causing some inhibition at lower substrate concentrations, but causing an indirect activation of HMT at higher histamine concentrations owing to reduction of substrate inhibition. This effect of H1 antihistamines on HMT has previously been observed by ourselves (Francis, Thompson & Greaves, 1980) and others (Taylor & Snyder, 1972). Whether these effects of oxatomide on HMT activity are likely to be clinically significant remains uncertain.

Our results confirm that oxatomide possesses potentially useful H1 antihistaminic activity, although like other H1 antihistamines it appears to possess weak anti-muscarinic activity as well. We have been unable to confirm claims that oxatomide can suppress evoked histamine release. These and other aspects of the pharmacology of oxatomide need to be followed up by *in vivo* studies, preferably in the human subject, since in this situation solubility of oxatomide may be much less of a problem.

ACKNOWLEDGMENTS

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Case Reports

Nystatin-induced fixed eruption

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SUMMARY

A patient is reported who developed a typical fixed drug eruption after ingestion of a single tablet of nystatin.

Nystatin is not a common cause of allergic reactions, although occasional cases of contact dermatitis (Wasilewski, 1970; Coskey, 1971; Coskey, 1978), fixed drug eruption (Kandil, 1969) and pustular reaction (Petrozzi & Witkowski, 1971) have been reported. This communication describes a case of fixed eruption provoked by nystatin.

REPORT OF A CASE

A 30-year-old single male attended the clinic, complaining of a skin rash, pain in joints and fever of 2 days duration. No history of any drug being taken was recorded except one tablet of nystatin 2 days previously. He gave a history of an urticarial rash from aspirin, paracetamol and penicillin, and generalized erythrodermia from oxytetracycline, septrin and minocin. He had reacted on separate occasions to each of these drugs.

The patient looked ill with a temperature of 37·8°C. Cutaneous examination showed six typical lesions of fixed drug eruption—well defined hyper-pigmented macules, dusky red in colour, circular in shape, varying in size from 1–1·4 cm, involving the forehead, trunk, palms and wrist (Fig. 1).

A patch test was performed with nystatin (Mycostatin) ointment which showed no reaction after 48 h. A provocation test was performed with one tablet of nystatin (500,000 units). After 2 and 8 h, blood samples were taken to determine the plasma concentration of nystatin, which was less than 1 μg/ml in both. A severe fixed eruption was observed 8 h after the drug administration, the lesions recurring in exactly the same sites. Complete recovery occurred slowly. The patient was instructed not to take nystatin again.

COMMENT

Allergic reactions to systemic administration of nystatin are rare. This may be attributed to the fact that the drug is absorbed in insignificant quantities from the intestine. In earlier reports, blood concentrations of 2·4 and 7·5 μg/ml were observed following oral administration of 6·75–10 million units

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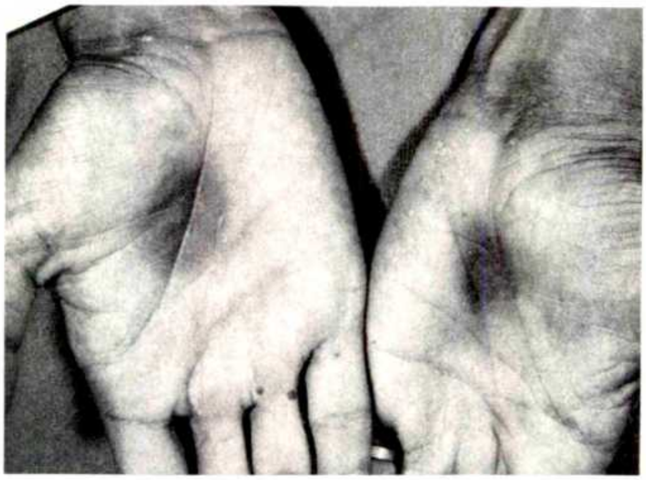

FIGURE 1. Palm of the hands showing fixed eruption due to nystatin.

(Tarbet & Sternberg, 1954; Newcomer *et al.*, 1955, 1960). In the present study plasma levels were less than 1 μg/ml. However, it is known that poorly absorbed drugs such as phenolphthalein (Lewin, 1962), sulphasuxidine and sulphathalidine (Tan & Copeman, 1974) are capable of causing fixed eruption. Kandil (1969) also demonstrated the ability of nystatin to produce a fixed drug eruption in a patient previously sensitized with amphotericin B. Thus the possibility, howsoever remote, of fixed eruption from nystatin should be kept in mind.

ACKNOWLEDGMENTS

I am indebted to the director of King Abdulaziz Teaching Hospital for permission to report this case and my thanks are due to E.R.Squibb and Sons Ltd., for analysing the nystatin level in blood samples.

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Primary cutaneous aspergillosis in a premature neonate

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SUMMARY

A case of primary cutaneous aspergillosis in a 3-week-old premature infant is reported. The lesion, which presented as a solitary nodule surmounted by three pustules, occurred following the use of elasticized and cloth adhesive tape and was cured by surgical excision. Factors predisposing to this infection included maceration, antibiotic usage, and prematurity.

Opportunistic fungal infections are of increasing importance in the immunocompromised host (Young *et al.*, 1970; Rose & Varkey, 1976; Prystowsky *et al.*, 1976). Primary cutaneous aspergillosis has been reported previously both in immunocompromised patients and in individuals apparently without an associated illness (Myers & Dunn, 1930; Cahill, El Mofty & Kawaguchi, 1967; Caro & Dogliotti, 1973). We report here a case of primary cutaneous aspergillosis in a premature neonate.

CASE REPORT

The patient was an 800 g girl born after an uncomplicated 26 week gestation. Moderate respiratory distress was noted immediately, resulting in transfer to the neonatal intensive care unit at the Massachusetts General Hospital. There was no family history of immuno-deficiency. Physical examination on admission revealed a dusky premature baby girl in respiratory distress with grunting, flaring of the nostrils, and retractions. The skin was unremarkable. A ductal murmur was noted. The admission haematocrit was 38·5%, white cell count 14,000/mm^3 with 49% neutrophils, 9% bands, 23% lymphocytes, and 14% monocytes. Chemical investigations were unremarkable except for a total bilirubin of 5·9 mg per decilitre.

As a precaution against sepsis the baby initially received ampicillin (100 mg/kg/day) and gentamicin (5 mg/kg/day). These antibiotics were discontinued after 72 h when blood cultures drawn at birth proved negative.

Ligation of the patent ductus arteriosus was performed on day 7 of life. Postoperatively, a left

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chest tube, held in place by an elasticized adhesive, was present for 2 days during which time a prophylactic course of oxacillin (200 mg/kg/day) was given. The oxacillin was discontinued when the chest tube was removed and resumed on day 11 to prevent complications of an umbilical venous catheter placed at birth. The umbilical catheter was removed and antibiotics were discontinued on day 12.

On day 14 a fluctuant nodule on the left mid-back distant from the entry point of the chest tube was noted. The lesion was freely moveable over the ribs. The area of the nodule had previously been covered with the elasticized (Elastoplast) and cloth adhesive tapes used to stabilize the chest tube. Gram stain of material aspirated from the lesion showed polymorphonuclear leukocytes without bacteria. Repeat stain was interpreted as showing a few Gram positive cocci. The patient was started on cefamandole (80 mg/kg/day) while culture of the aspirate was incubating. This culture grew only 'few *Aspergilli*,' which were considered to be a contaminant. There was no bacterial growth. Cefamandole was discontinued after 48 h.

On day 22 dermatology consultation was requested. The lesion at that time was a 1 cm slightly erythematous fluctuant nodule capped by three tiny pustules. One-half millilitre of yellow-green material was aspirated from the lesion. Gram stain showed only polymorphonuclear leukocytes without bacteria. Swartz-Lamkins stain (Swartz & Lamkins, 1964) revealed wide, septate and non-septate branching hyphae with a few budding forms. The culture grew *A. flavus* and no bacteria. Amphotericin B was withheld since the baby did not appear septic and its use would have compromised the baby's fluid status and calorie intake. The lesion was definitively treated by excision on day 24. Histopathological examination revealed necrotizing granulomas and sinus formation with invasion of tissue by fungal elements consistent with *Aspergillus* (Fig. 1). At one area granulomatous inflammation extended to a margin, otherwise the margins were clear. Culture from the biopsy specimen grew *A. flavus*.

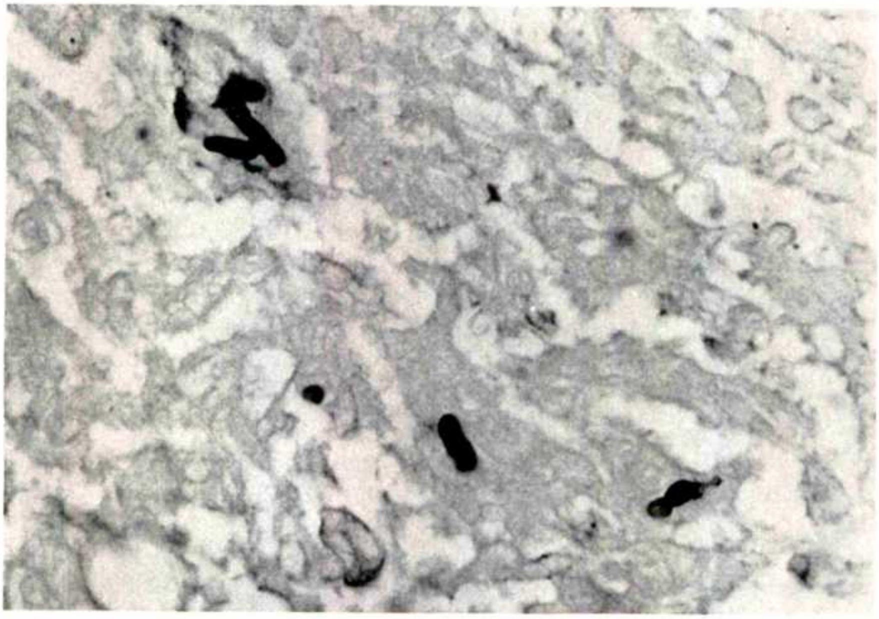

FIGURE 1. Photomicrograph demonstrating fungal elements within the dermis (Methenimine silver stain—400 ×).

On day 38 cardiac catheterization was performed to evaluate the aetiology of recurrent pulmonary oedema. The procedure was complicated by cardiac arrest and resuscitation efforts were unsuccessful. No evidence of systemic or local *Aspergillus* was present at post-mortem examination.

DISCUSSION

Aspergillus species have been most commonly associated with allergic pulmonary disease. These organisms have also been associated with invasive disease in lung, gastro-intestinal tract, central nervous system, genitourinary tract, kidney, thyroid, heart, sinuses, diaphragm, eye, bone, and skin (Landau, Newcomer & Schulz, 1963; Young *et al.*, 1970). The lesion in this case arose at a site previously covered with an elasticized adhesive (Elastoplast) and cloth adhesive tape. Prystowsky *et al.* (1976) reported four cases of cutaneous aspergillosis. Two of these patients had haematological neoplasms and two had aplastic anaemia. In three of these patients the lesions arose at a site in contact with paper covered boards or adhesive tape securing intravenous infusion sets. Mead *et* al. (1979) reported a case of cutaneous *Rhizopus* infection associated with an elasticized adhesive at the site of the lesion. They cultured 85 rolls of elasticized adhesive from their institution; 32 fungal isolations were made, 13 were *Aspergillus* species and eight of these were *A. flavus*. Two mechanisms by which tape may enhance infectivity of fungal organisms are: (1) maceration due to occlusion, providing a receptive local environment; and, (2) damage to the barrier function of the skin during tape removal, thus opening a route of infection.

The most important factor predisposing towards infection in this case was prematurity. Gotoff (1974) concludes that the immunological apparatus of the young infant is deficient when compared with that of the older child or adult; studies suggest a quantitative deficiency of chemotactic activity for polymorphonuclear leukocytes in sera of newborns, decreased ability of cord serum in pre-term infants to support phagocytosis, and generally a less vigorous antibody response to immunizing agents or naturally occurring infections in the neonate compared with the older child or adult. It is also noted that delayed hypersensitivity skin tests are more difficult to elicit in the newborn.

Antibiotic use as a predisposing factor in opportunistic fungal infection has been debated. Our patient was on oxacillin in the days before observation of the lesion. Young *et al.* (1970) pointed out that while many reports note the onset of myocoses in patients being treated with multiple antibiotics, the patient population prone to fungal infection is usually on antibiotics for some other reason. Bodey (1966) found in acute leukaemia that antibiotics were no more frequently administered to patients developing fungal infections than to those without them. It was noted, however, that antibiotic administration did increase the number of patients from whom *Candida* could be cultured. The same observer noted in 1975 (Bodey, 1975) that antibiotics increase the susceptibility of experimental animals to fungal infection and that superficial *Candida* dermatitis is most prevalent in patients receiving antibiotics.

It is important to note that primary cutaneous aspergillosis can occur in the premature neonate. There was no evidence of systemic aspergillosis in this case. Ante mortem cultures of blood, cerebrospinal fluid, and sputum as well as post-mortem cultures of blood, lung, and brain failed to grow any fungus. In this case the possibility exists that elasticized adhesive or cloth adhesive tape was the source of the organism.

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Systemic Weber-Christian disease: a case report with lipoprotein profile and immunological evaluation

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SUMMARY

A case of fatal systemic Weber-Christian disease is described. *In vitro* immunological function and lipoprotein measurements are presented with a subsequent discussion on the possible aetiology of the condition.

Relapsing nodular febrile non-suppurative panniculitis was first described by Pfeifer in 1892 but only subsequently named in this way when further cases were described by Weber in 1925 and Christian in 1928. The eponym 'Weber-Christian disease' was introduced by Brill in 1936. Since that time about 150 cases have been reported although only fifteen with confirmatory autopsy findings. It is proposed to follow the suggestion of Milner & Mitchinson (1965) that the name 'systemic Weber-Christian disease' be applied when the lesions occur in adipose tissue other than the panniculus adiposus and that the term 'Weber-Christian panniculitis' be used when the lesions are confined to the subcutaneous adipose tissue. The case described is one of interest since it is the first time that the lipoprotein profile and investigations of the immune status have been reported.

CASE HISTORY

A 27-year-old caucasian woman was referred to medical outpatients in 1973 complaining of diarrhoea and rectal bleeding. History and examination showed her to be otherwise well. Barium studies revealed striking loss of haustration in the whole colon with an abnormal mucosal pattern in the transverse and iliac colon and a grossly deformed ileo-caecal valve. There was a constant slight dilatation of the terminal ileum. The X-ray findings taken in conjunction with normal sigmoidoscopic appearances and a normal rectal biopsy led to a presumptive diagnosis of Crohn's disease. Treat-

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ment was commenced with sulphasalazine (0·5 g) and codeine phosphate (60 mg) three times daily. She was entered into a double blind two dosage schedule trial of azathioprine in Crohn's disease and drew the low dose, receiving only 12·5 mg/d from June 1973 to November 1974. Her symptoms improved and no other treatment was given. Repeat barium studies in September 1974 confirmed the previously noted abnormalities but since the symptoms had settled all treatment was discontinued in November 1974.

During this period she had been treated briefly with amytriptyline 10 mg three times daily for mild depression and received sclerosing injections on three occasions for superficial varicose veins. In May 1974 she had a vaginal termination of pregnancy.

From November 1974 until June 1975 she was completely asymptomatic but in July she was admitted as an emergency because of diarrhoea and rectal bleeding. This apparent recurrence of Crohn's disease was treated symptomatically and again her condition improved. In September 1975 she was readmitted with weight loss, pyrexia and a skin rash. This was composed of pigmented discoid indurated lesions on arms, legs and lower back. Biopsy of one of these showed an extensive subcutaneous panniculitis with both acute and chronic inflammatory cells and a few foam cells (Fig. 1). There was no vasculitis but there was some perivascular accumulation of mononuclear cells in both the subcutaneous tissue and the lower dermis. Although the inflammatory changes affected fat lobules as well as septa the changes were interpreted at that time as erythema nodosum, possibly related to the Crohn's disease. Other investigations showed a mild anaemia (Hb 11·29 g/l) and leucopenia ($3{\cdot}8 \times 10^9$ cells/l). Liver scan showed a patchy liver uptake and moderate splenomegaly.

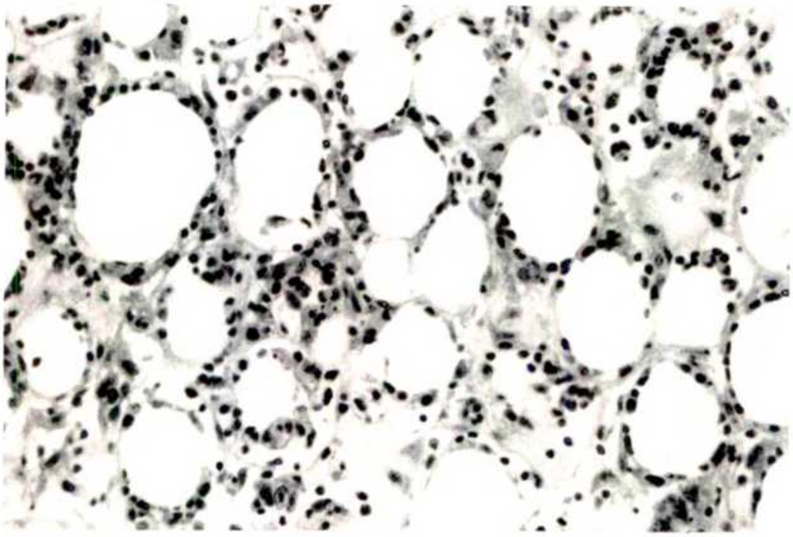

FIGURE 1. September 1975. Biopsy of subcutaneous tissue showed an extensive diffuse panniculitis in which chronic inflammatory cells were predominant (H & E × 320).

Abdominal lymphoma was now considered to be a serious possibility and thus a lymphangiogram was performed, which showed generalized abdominal lymphadenopathy. A laparotomy was undertaken on October 1975. The spleen was noted to be enlarged (565 g) and was removed. There was slight hepatomegaly but nothing to suggest lymphoma. The small and large intestine also appeared externally normal. Histological examination of the spleen and several abdominal lymph nodes showed reactive changes only and the liver biopsy was substantially normal.

A presumptive diagnosis of a 'collagen disorder' was made and the patient commenced on prednisolone (30 mg/d). She improved considerably, the rash faded and she returned home, taking 20 mg prednisolone/d. However there was a recurrence of pyrexia and painful lumps on her legs in December 1975. She also had increasing hepatomegaly at this time. Over the ensuing months her condition gradually progressed with more frequent episodes of pyrexia and general ill-health despite continuous steroids.

In March 1976 the rash which had affected the extensor aspect of the left elbow and the posterior aspect of both legs was noted to have faded and caused scarring and induration. In June 1976 she had to be readmitted with a high swinging fever, the terminal part of which is shown in Fig. 3. She developed jaundice for the first time and her liver function tests, which had previously been normal, showed a total protein of 60 g/l; albumin, 20 g/l; bilirubin, 98 μmol/l; alkaline phosphatase of 1200 u/l and an SGPT of 100 u/l. Other investigations revealed a coagulation disorder with a prothrombin time of 18·5 s compared to a control of 14 s, a kaolin partial thromboplastin time of 38 s with a control of 35 s and a thrombin time of 25 s with a control of 15 s. Measles and rubella antibody titres were raised as shown in Table 1. The toxoplasma dye test was positive at 1:8, the significance of which is not understood. Fasting blood lipids were measured on three occasions during July 1976 and were found to be elevated with a cholesterol of 8·2 mmol/l and triglycerides of 14·0 mmol/l. Lipoprotein electrophoresis demonstrated a Frederickson Type I hyperlipidaemia. Other investigations were unremarkable except for a mildly positive immunofluorescent antibody to smooth muscle on one occasion at a dilution of 1:50.

TABLE 1. Virus and bacterial antibody titres

	Epstein-Barr virus antibody					
	Anti IgM	Anti IgG	Rubella IgG	Measles	Toxoplama Dye test	ASO titre
October 1975	<10	40	1:64	—	1:32	100
March 1976	<10	—	1:4000	1:4000	—	—
July 1976	<10	160	1:1024	1:1024	1:8	1000
August 1976	<10	160	1:8000	1:256	1:32	1000

Negative Antibody Titres were as follows: Q fever; *M.pneumoniae*; Psittacosis; *Salmonella Typhi* H,O, *paratyphi* H,O, *Brucella abortus*; *Leptospirosis ictohaemorragica*, *canalicola*, *hebdomadis*; W.R.; V.D.R.L.; hepatitis B core antigen; hepatitis B surface antigen.

The immune status of the patient was evaluated in July 1976 when she was receiving 30 mg of prednisolone daily, A.C.T.H. 40 units daily and systemic antibiotics. She was found to be blood group A rhesus positive with an HLA type A1 B8 homozygous. Haemoglobin was slightly low at 11.2 g/l with a normal white count of 8·2 cells × 10^9/l and an ESR of 14. The differential white count was normal with 6·1 neutrophils, 1·8 lymphocytes, 0·2 monocytes and 0·1 eosinophils with no other cells seen (counts expressed as cells × 10^9/l). A neutropenia had been briefly observed while an in-patient in October 1975 when the total white count fell to 2·1 cells × 10^9/l.

In view of the abnormal liver function tests a liver biopsy was performed under fresh frozen plasma cover. This showed a normal hepatic architecture but there was accumulation of fat in periportal liver cells. In some there had been a coalescence of lipid droplets to form quite large vacuoles which were often surrounded by a rim of mononuclear cells but foam cells were not conspicious at

TABLE 2. Results of immune status and lipoprotein profile

Hb: 11·3 g/l. Total white count: 8·2 × 10^9/l
ESR 9 mm at 1 h
HLA Type: HLA A1 B8 Homozygous
Fc rosette forming cells (B): 17%. Blood group A, rhesus positive
Sheep red blood cell rosette forming cells (T): 71%
PHA Transformation:
  stimulation index = 75 (normal 57–350)
  maximum PHA response = 5 μg/ml (normal 0·6–5·0 μg/ml)
PPD Transformation:
  stimulation index = 3 (normal 0·8–40)
  maximum PPD response = 65 μg/ml (normal control 10 μg/ml)
  (level compatible with tuberculin negative subject)
Immune complexes: strongly positive by C_{1q} binding and by polyethylene glycol complement consumption.
Complement: CH_{50} 2080 u/ml (normal 900–1800 u/ml)
  C3 90 mg/l (normal 60–180 mg/l)
Immunofluorescent antibodies to cell nuclei, mitochondria, smooth muscle, parietal cells, thyroid microsomes and thyroid colloid negative except positive at 1:50 on one occasion to smooth muscle.
Rose-Waaler Test negative   Cryoglobulins negative
Cholesterol 8·2 mmol/1 (normal 3·6–6·2 mmol/l)
Triglycerides 14·0 mmol/l (normal 0·6–1·7 mmol/l)
Lipoprotein Electrophoresis, Frederickson Type-1

this time. Most portal tracts contained an intense infiltrate of plasma cells and lymphocytes. Centrilobular cells were more normal (Fig. 2a). At the centre of a simple lobule foam cells could be seen apparently penetrating the wall of a central vein to reach the lumen (Fig. 2b). The liver biopsy appearances were thus quite unlike those of the liver of chronic active hepatitis and the diagnosis remained uncertain.

She finally developed a relapsing intermittent fever which responded neither to increased steroids nor to antibiotics (Fig. 3). There was evidence of progressive liver failure with increasing jaundice, the development of a liver flap and deterioration of her coagulation tests. Despite intensive supportive therapy she died of liver failure in September 1976.

AUTOPSY

The body was deeply jaundiced. There was fairly extensive blotchy purpura of the trunk and limbs with pigmented scars of the lower legs and forearms. The liver was markedly enlarged, had a smooth capsule and a blotchy greenish brown surface with scattered petechiae. The results of the previous laparotomy were noted but otherwise the intra-abdominal lymph nodes, previously found to be enlarged, and the vital organs all appeared macroscopically normal.

Microscopically the liver changes were very similar to those in the last biopsy. There was striking periportal fatty change with some very large fat vacuoles some of which now contained foam cells. There was also patchy eosinophilic necrosis and accumulation of a few mononuclear cells. The centrilobular liver cells were more normal with only fine droplet fatty change but here the sinusoids were distended by numerous large foam cells which were also present in the lumina of a number of central veins. The sternal marrow showed rather similar pathology with circumscribed foci of eosinophilic necrosis which were associated with many foam cells (Fig. 4). Foam cells without

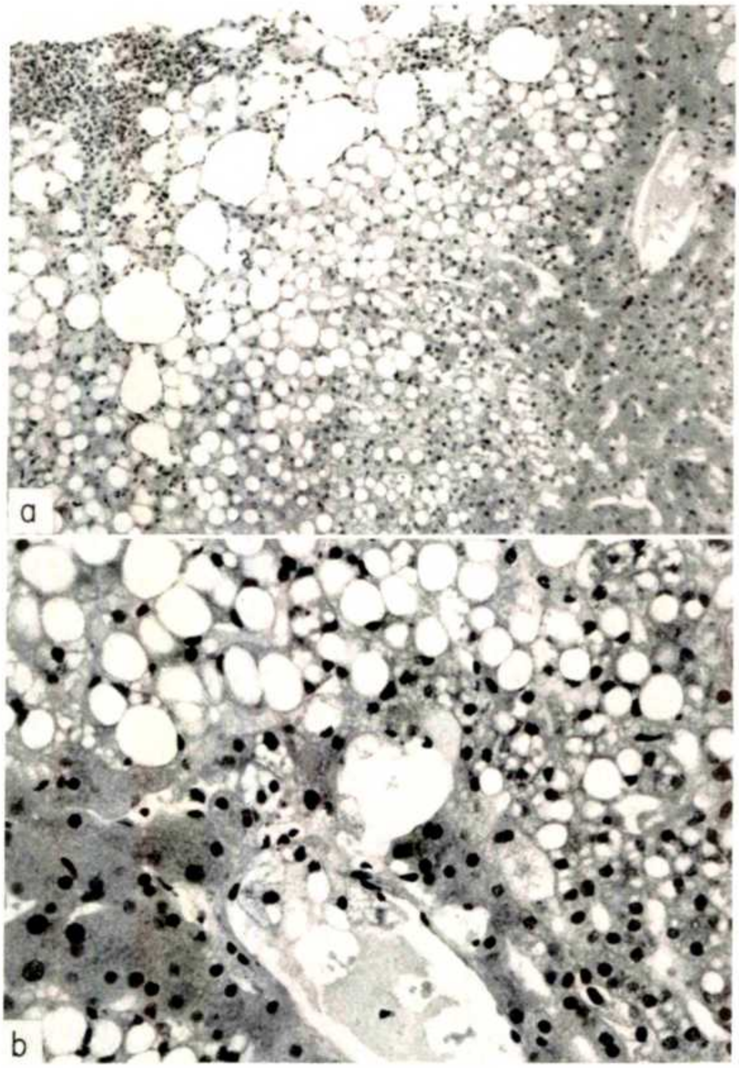

FIGURE 2. (a) June 1976. Liver biopsy showed extensive periportal fatty change with large fat vacuoles, many ringed by mononuclear cells, adjacent to the portal tracts. These contained mixed chronic inflammatory cells. Liver cells at the centres of the lobules were more normal (H & E × 128). (b) Detail of (a) to show penetration of the wall of a central vein by foam cells (H & E × 320).

necrosis were found in adipose tissue from the small bowel mesentery, periadrenal and retroperitoneal regions, but not in the thorax. Subcutaneous tissue was not sectioned at post mortem.

Retrospective review of the skin and subcutaneous tissue biopsies taken in September 1975 confirmed that the appearances were those of a panniculitis involving lobular adipose tissue as well as interlobular septa and were very similar to those in the liver and bone marrow. At all three sites the morphological appearances are characteristic of Weber-Christian disease.

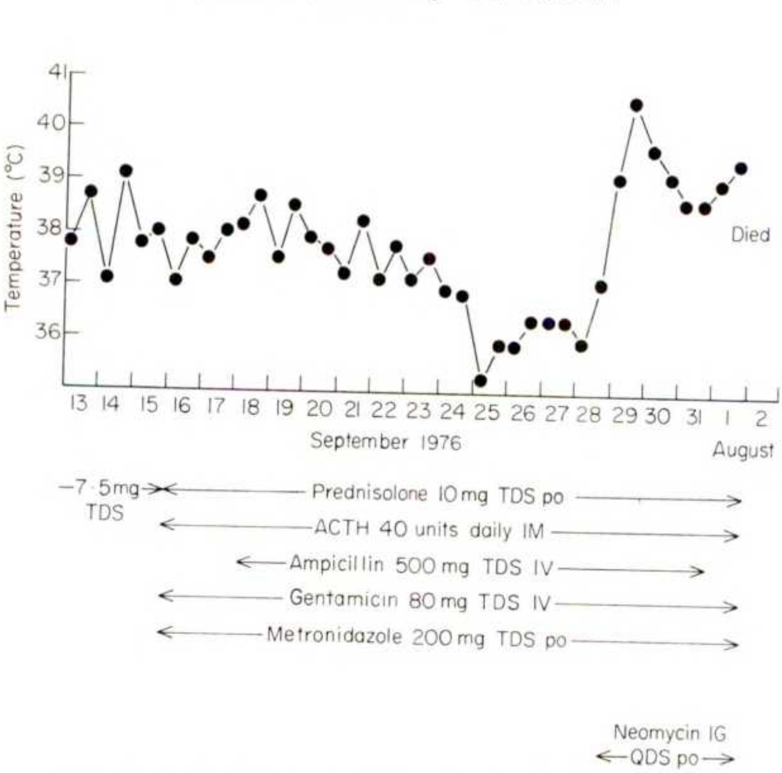

FIGURE 3. Terminal T.P.R. Chart showing drugs administered.

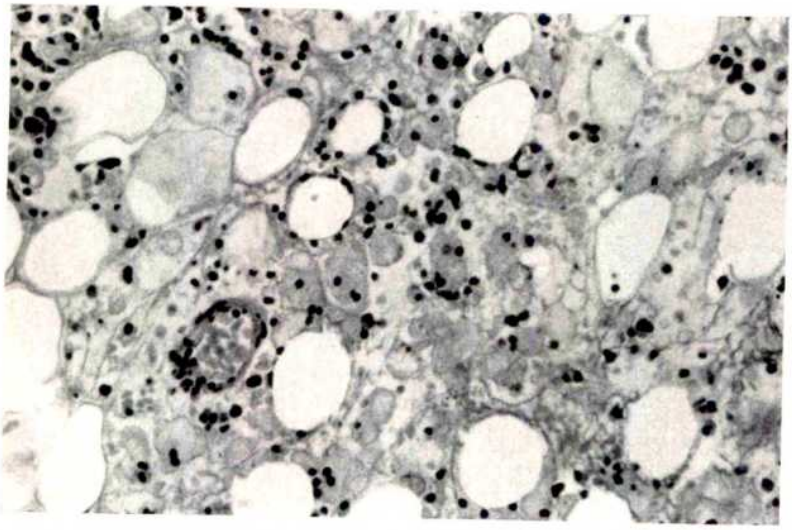

FIGURE 4. Post-mortem, sternal marrow. Panniculitis with numerous foam cells (H & E × 320).

DISCUSSION

The pathology of Weber-Christian panniculitis has been clearly defined and the diagnosis is confirmed by histological examination of subcutaneous tissue which shows pleomorphic inflammatory cellular infiltration of the subcutaneous lobular adipose tissue and interlobular septa with sparing of the overlying dermis and epidermis. The walls of small vessels are sometimes infiltrated by inflammatory cells or are necrotic. The infiltrate usually consists mainly of large mononuclear cells, including fat filled macrophages and sometimes giant cells. Polymorphonuclear leukocytes are not usually a conspicuous component except in the earliest stages. Necrosis of local adipose tissue accompanies the inflammation and is usually followed by fibrous repair. Whilst lobular panniculitis may be seen in other conditions, the combination of lobular panniculitis containing numerous foamy macrophages with the characteristic clinical picture is usually accepted as diagnostic of Weber-Christian disease. Diagnosis of internal lesions is more difficult and in this case was confirmed only by microscopic examination at autopsy.

The clinical presentation of systemic Weber-Christian disease varies and depends on the degree and site of internal lesions. Anaemia has been reported as well as infiltration of the bone marrow (DeLor & Martz, 1955), as seen here. Patchy shadowing of the lungs due to infiltration occurs and perforation of the bowel has also been described (Schoen, Reingold & Meister, 1958). The most common presentation is malaise, abdominal discomfort, tiredness and loss of weight in the presence of the subcutaneous lesions (Milner & Mitchinson, 1965). Hepatomegaly with or without splenomegaly is a common finding. Differential diagnosis was reviewed by Förström & Winkelmann (1976) who felt that the disease is more common than is realized and may go undiagnosed. Investigation should include a thorough search for signs of bacterial or virus infection, hypersensitivity to bacterial antigen, autoallergic disease and malignancy.

Frederickson type I hyperlipidaemia is itself relatively rare and thus it seems possible that the link with systemic Weber-Christian disease may be more than coincidental. Serum lipoproteins were not measured in the previously reported cases and thus it will be important to pursue this relationship in future.

The *in vitro* immunological investigations have shown a mildly elevated total haemolytic complement, a low T cell response to purified protein derivative, high levels of circulating immune complexes and a terminal non-specific rise in antibody titres, the latter probably due to the associated liver failure. These, together with progressive infiltration of adipose tissue with mononuclear cells and fat-filled macrophages in the presence of hypertriglyceridaemia, all point to an abnormality of the immune system as proposed by Allen-Mersh (1976). It is impossible to draw any conclusion from the isolated finding that the HLA type was A1 B8 homozygous, but it will be of interest to look at the HLA status in future cases.

The combination in this case of high levels of circulating immune complexes with a Frederickson type 1 hyperlipidaemia points to an immune or autoimmune disease of adipose tissue as the aetiology of this condition.

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Lawrence-Seip syndrome

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SUMMARY

A case is reported showing insulin resistant diabetes mellitus with acanthosis nigricans, clitoral hypertrophy and the typical facies of the Lawrence-Seip syndrome.

The Lawrence-Seip syndrome is a genodermatosis inherited as an autosomal recessive. The main features are acanthosis nigricans, curly hair, generalized hypertrichosis, loss of subcutis and facial fat giving a gaunt appearance, marked musculature with protruberant abdomen, and insulin resistant diabetes without ketosis (Cairns, 1972).

CASE REPORT

A 16-year-old girl born to consanguinous parents presented with pigmented thickened itchy skin lesions over the neck, axillae, hands, groin and feet since early childhood. There was no history of polyphagia or polydypsia but there was a history of polyuria. Her first menstrual period was at the age of 15 years. After that she had had no periods at all.

On examination she had the typical gaunt facial appearance due to loss of fat, excess of lanugo hair over the face (Fig. 1) and curly hair over the scalp. In addition hyperpigmented, verrucous and velvety plaques of acanthosis nigricans were present over the face, nape of the neck (Fig. 2), axillae, forearms, legs, feet, groin and abdomen and also mucous membranes of the tongue, vagina and vulva. Gynaecological examination revealed a hypertrophied clitoris (Fig. 3) and hypoplastic uterus. Ophthalmic examination did not show any diabetic lesion. Psychiatric assessment showed moderate mental retardation with an Intelligence Quotient of 53 using Gesell's criteria. There was no hepatosplenomegaly or neurological abnormality.

Complete blood count, chest X-ray and electrocardiogram were normal. Contrast radiography of the gastrointestinal tract and endoscopy to exclude associated malignancy were normal. Skin biopsy from a pigmented and thickened area was consistent with acanthosis nigricans. Biopsy of an atrophic skin area was not diagnostic of lipoatrophy.

A marked feature was persistent glycosuria with co-existent hyperglycaemia but without ketosis.

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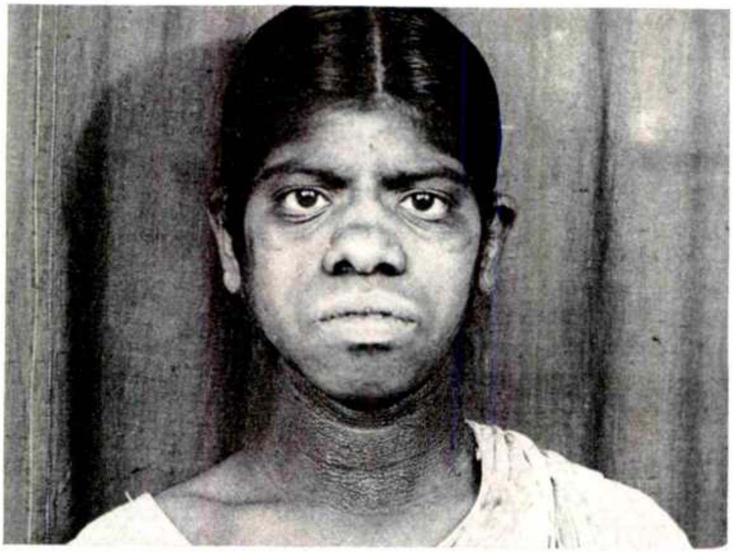

FIGURE 1. Gaunt appearance of the face with acanthosis nigricans over the neck.

Oral glucose tolerance test showed moderate to severe diabetes. Random blood sugar estimations at frequent intervals varied from 284 mg% to 580 mg% without any specific treatment. Other investigations showed: serum triglycerides 160 mg%; serum cholesterol 137 mg%; urinary oestrogen 14·7 μg/24 h; urinary 17-ketosteroids 24 mg/24 h; growth hormone assay and insulin assay within normal limits.

The patient was started on plain insulin 20 units twice daily. The doses were stepped up to 100 units eight hourly, but the glycosuria and hyperglycaemia persisted.

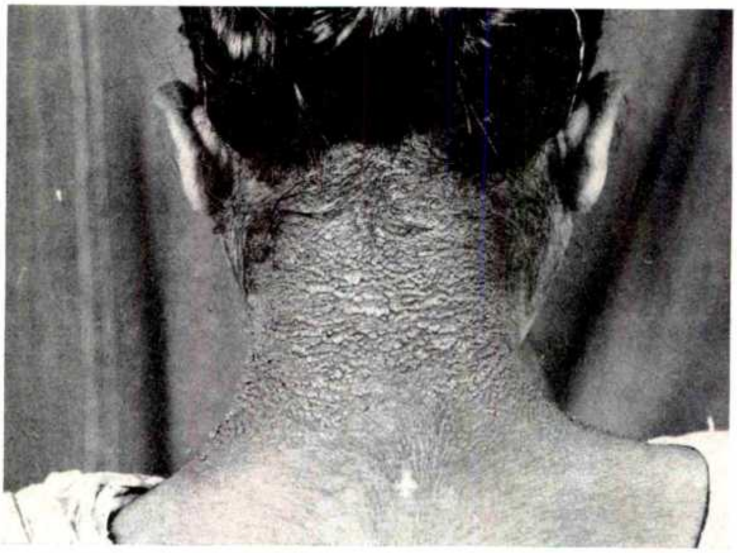

FIGURE 2. Verrucous plaques over the nape of the neck.

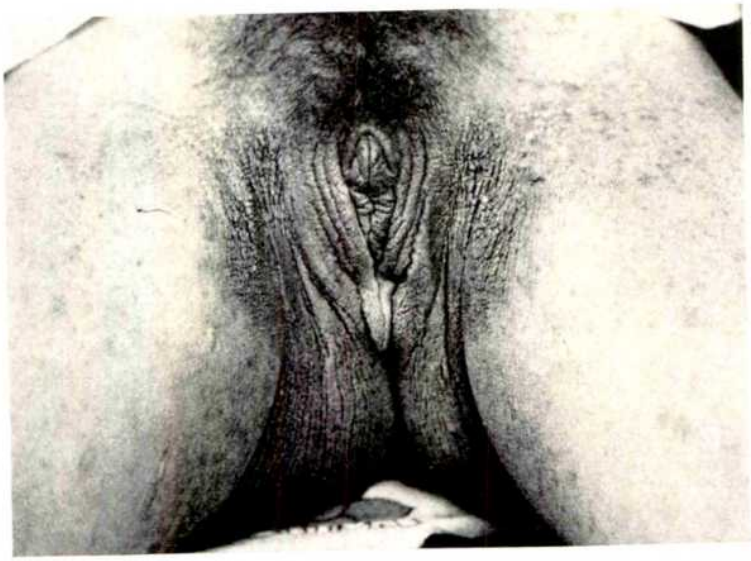

FIGURE 3. Clitoral hypertrophy with acanthosis nigricans lesions on either side.

Palmar dermatoglyphics using the standard Ink method revealed prominent ridges, ulnar loop pattern (U) in all the finger tips of the right palm and in the finger tips of the index and little fingers of the left palm, whorl pattern (W) in the finger tips of the thumb, middle and ring fingers of the left palm, large loop pattern (L) in I_4 area of both palms, a raised total finger ridge count (TFRC-183) and normal position of axial triradius in both palms. The well defined ridges, large loop pattern in I_4 area and a raised TFRC may be of significance but more cases have to be studied.

Along with plain insulin the patient was given 50,000 i.u./day vitamin A orally and keratolytic ointment topically for her skin lesions and there was some improvement after 3 months.

DISCUSSION

In 1946, Lawrence first described a syndrome which he named acquired lipoatrophic diabetes characterized by loss of subcutaneous fat, insulin resistant diabetes without ketosis, hepatic cirrhosis and hyperlipaemia (Fleischmajer & Matus, 1975). Seip described a congenital syndrome similar to that of Lawrence but without diabetes and with rapid growth, advanced bony age, hepatomegaly, hypermusculature, hyperpigmentation and corneal opacities (Domonkos, 1971). Clitoral or penile enlargement was apparent in early life, as in Reed's observation (Reed *et al.*, 1965). Seip and Trygstad (1963) found moderately raised 17-ketosteroids in their five cases and increased growth hormone in three of their five cases.

This patient had most of the typical featurs of the Lawrence-Seip syndrome. Other features of the syndrome like cutaneous xanthomata, corneal opacities, renal, cardiac, and ovarian anomalies and hepatosplenomegaly were not present.

According to Flier, Kahn & Roth (1979), in patients with reduced sensitivity to insulin, a normal biological effect to that hormone can be obtained with supernormal blood insulin levels. This can be achieved by stimulating endogenous secretion or exogenous therapy. However, in some patients massive amounts of insulin fail to produce a biological effect and the spectrum of insulin resistance varies from normal glucose tolerance to severe diabetes. This patient with severe glycosuria, persistent hyperglycaemia and a normal plasma insulin level may fit in with type B insulin resistant syndrome of Flier's series in which 10 of their 14 cases had extreme resistance and acanthosis nigricans. The normal plasma insulin assay in this case may be attributed to the mechanism of insulin resistance operating at or beyond the level of hormone receptor interaction (Flier *et al.*, 1979). Hamwi *et al.* (1966) found normal plasma levels of insulin in one case and in that case they also found a potent lipid mobilizing and insulin antagonistic substance in the urine.

Acanthosis nigricans as part of the syndrome is emphasized in this report. Most standard reports on acanthosis nigricans emphasize the existence of only the juvenile type and the adult type associated with malignancy. Little emphasis is made on acanthosis nigricans as part of more complex syndromes.

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Comment

Is epidermal homeostasis a necessity?—comments on epidermal growth control

This review was stimulated by the author's dissatisfaction with current views on mammalian epidermal homeostasis. A comprehensive review of the topic will not be attempted; instead, it is intended to survey the possibilities and submit some ideas for examination.

'Epidermal homeostasis' has come to mean maintenance of epidermal population size, though it would seem more reasonable for the organism to be concerned with maintenance of function than constancy of structure. Nonetheless, let us consider the problem from the structural standpoint.

Most discussion on this topic starts from the premise that there is one (or several) mechanism(s) responsible for epidermal homeostasis. It is often suggested that it is self evident that the constancy of epidermal structure and its rapid restitution after injury are evidence enough of a strong system of epidermal control. This premise should be critically re-examined before yet more detailed studies are mounted to attempt the isolation of 'growth control substances' from biological sources.

Dissociated human epidermal cells form stratified squamous epithelial tissue after some 2 weeks in culture (Rheinwald & Green, 1975; Green, 1977). This 'epidermis' maintains an essentially normal pattern of differentiation with mature 'corneocytes' sloughing off at the surface. Although the presence of fibroblasts is required in the culture flask it is not clear whether this is because they provide a suitable physical substratum or secrete some essential growth promoting substance. The lack of systemic influences and absence of blood vessels, neural elements, epidermal dendritic cells and surrounding epidermis imply that the epidermal cell itself is able to develop and retain an orderly adult type of epidermal structure.

The fact that the 'orderly structure' of the epidermis is so easily abrogated by a variety of comparatively minor insults might also indicate that the control mechanism (if it exists) can be easily 'overruled' (at least temporarily). For example, the concerted and purposive series of alterations to epidermal structure found in transepidermal elimination (Mehregan, 1970), induced by a relatively minor stimulus, (Kirsch & Hukill, 1977; Bayoumi, Gaskell & Marks, 1978) suggests that at the best the control exerted is weak. If epidermal cells were genetically programmed to divide at a constant rate, and had the capacity to respond to stimuli with a burst of mitotic activity, rapidly reverting to their inherent generative capacity, would this not meet most of the mitotic requirements of the epidermis, and indeed fit most of the observations?

CHEMICAL REGULATION

In the 1950's and 1960's the concept of 'negative feed back control' was borrowed from cybernetics and used to explain many biological phenomena involving 'regulation'. Weiss & Kavanau (1957) used the concept to explain growth control and of course the older chemical law of mass action would appear to fit in with this philosophy. In the 1960s Bullough & Laurence and others originated and developed the 'chalone hypothesis' (Bullough & Laurence, 1960a, 1960b, 1961; Bullough, 1962). It was

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suggested that the mature cells of constantly dividing tissues perpetually produced tissue specific (but not species specific) inhibitory substances that prevented unrestrained mitotic activity. The inhibitory substances were termed 'chalones' (derived from the Greek meaning to slow down). Evidence was produced implicating adrenalin in the action of chalone and favouring a chalone-adrenalin complex as the effector substance (Bullough & Laurence, 1964, 1966). The early papers from the Bullough group reported that hydrocortisone also contributed to the chalone effect, but later work was able to demonstrate an inhibitory action of preparations of chalone without hydrocortisone. Reports from other groups appeared, which affirmed the presence of an inhibitory material in skin extracts or homogenates (Chopra, Yu & Flaxman, 1972; Elgjo, 1974; Frankfurt, 1971). Some, however, were less encouraging about the presence of a chalone (Marks, Fukui & Halprin, 1971). The inhibitory 'chalone activity' seemed to be water soluble, heat labile and to be precipitated by the addition of alcohol (Bullough, Hewitt & Laurence, 1964). Attempts at chalone isolation and characterization were not successful, but it was suggested that it was a water soluble glycoprotein with a molecular weight of approximately 30,000 and an isoelectric point between pH 5·0 and 6·0 (Boldingh & Laurence 1968). It was later claimed that particular parts of the epidermis produced their own specific inhibitors or chalones, and a melanocyte chalone (Bullough & Laurence, 1968a) and a sebaceous gland chalone (Bullough & Laurence, 1970) were described.

'Chalone' activity has also been described in preparations from a variety of other tissues and cell types, including lymphocytes (Moorhead *et al.*, 1970), granulocytes (Rytomaa & Kiviniemi 1968), fibroblasts (Houck, Sharma & Cheng, 1973), lung parenchyma (Simnett, Fisher & Heppleston, 1969) and kidney (Simnett & Chopra, 1969). Even antichalones have been proposed (Elgjo & Edgehill, 1973). 'Epidermal chalone' containing extract was reported to have an inhibitory effect on tumour growth (Bullough & Laurence 1968b; Bullough & Deol, 1971) and this approach to cancer chemotherapy seemed to hold great promise. The site of action of chalones has never been adequately defined. However, recently two points of action on the mitotic cycle for epidermal chalone have been proposed on the basis of experimental evidence; G_1 and G_2 epidermal chalones (acting on the G_1 and G_2 phases) are believed to derive from suprabasal differentiating epidermis and basal epidermal cells, respectively (Elgjo, Laerum & Edgehill, 1972).

Chalones have titillated the biological imagination but not the critical faculties of researchers involved in epidermal growth control. Nonetheless, there are both theoretical and pragmatic reasons for being cautious about the epidermal chalone. The hypothesis of negative feed back control implies that the inhibitor material(s) is (are) produced by mature cells, i.e. replication is stopped by the chalone-product of the mature cell. This principle certainly applies to the endocrine system where the concentration of the active hormone product indirectly regulates its further production. However, the Malpighian cell (or for that matter the granular cell) is not 'mature', it is 'maturing'. The mature product of the epidermis is the constituent cell of the stratum corneum, the corneocyte. As modulation of desquamation may well take place within the granular layer and stratum corneum itself, teleologically it would seem 'inefficient' to regulate epidermal cell production by a substance (or substances) produced by a 'maturing' cell rather than by some function of the mature produce—the corneocyte.

The promise of a readily available non-toxic growth regulator material has lead several pharmaceutical companies, as well as numerous research groups outside industry, to attempt to isolate and characterize chalones. Some progress may have been made with lymphocyte chalone, but extraordinarily little with 'epidermal chalone' (for review see Houck, 1979; Allen & Smith, 1978). Clearly, such difficulty in the face of the combined resources of industry and academia and the isolation and characterization of other regulator substances, including pituitary peptides, endorphins and epidermal growth factor, could suggest to the more cynical that perhaps the material does not exist! To be fair the 'indicator systems' available to monitor the presence of chalone activity are grossly inadequate and this could be the partial explanation for the inability to produce pure preparations.

Anxiety about the chalone hypothesis also stems from the lack of adequate controls and the use of inappropriate methods by many of the workers in this field. Examples include failure to exclude a direct cytotoxic action of the tissue extract (or indirect cytotoxicity from complement activation), the use of thymidine incorporation as an index of DNA synthesis in whole skin organ preparations *in vitro* (Hansteen, Iversen & Refsum, 1979) and the failure to take account of the presence of thymidine (or thymidine kinase making exogenous thymidine inaccessible to cells) in the tissue extracts added to the culture media when employing thymidine incorporation techniques (Houck, 1979).

Glucocorticoid hormones damp down generative activity in a variety of tissues, including epidermis (Marks *et al.*, 1971; Marks & Williams, 1976). DNA synthesis within the epidermis correlates with the circulating level of T_3 (Holt & Marks, 1977) and is increased in acromegaly (Holt & Marks, 1976). Epidermal replication (at least in some anatomical sites) may also be affected by the ambient concentrations of steroid sex hormones (Shahrad & Marks, 1977; Mauvais-Jarvis, 1977) and appears to be sensitive to tissue levels of prostaglandins (Francis & Marks, 1977; Furstenberger & Marks, 1978) cyclic nucleotides (Voorhees *et al.*, 1973) and polyamines (Russell *et al.*, 1978). Epidermal growth factor can certainly stimulate epidermal growth experimentally, though it is not certain whether it has a regulatory role in the adult (Carpenter, 1978). Concentrations of calcium ions may also be important in modulating mitotic activity. If all these 'bio-materials' can affect the generative activity of the epidermis, does it not suggest that chalone control can at best be only marginally effective as a homeostatic mechanism? The original experiments which suggested negative feed back control to Bullough were based on the mitotic response to wounding (Bullough & Laurence, 1960a). One aspect of wounding that has never adequately been explained is 'post-wounding acanthosis'— the epidermal thickening that is seen at the wound margin and over the wound itself after re-epithelialization. On reflection this phenomenon seems an unlikely consequence of the re-establishment of chalone control as the likely overshoot would be toward epidermal thinning if anything. It could be better explained on the basis of 'an inherent epidermostat' attempting to reassert itself.

OTHER FACTORS THAT MAY REGULATE EPIDERMAL POPULATION SIZE

The epidermis of a fish does not form a stratified epithelium and does not differentiate into stratum corneum. The 'generative compartment' appears to be the entire tissue as DNA synthesizing cells occur throughout its thickness (Bullock, Marks & Roberts, 1978). How do cells 'escape' from this tissue so that its volume remains constant? There is at present no evidence for any particular mechanism, but it seems likely on consideration of other tissues that have no natural 'exit' for older redundant cells (such as hepatic tissue and endocrine tissues) that they die *in situ* and are phagocytosed, or are otherwise absorbed. This process is known as 'apoptosis' and has been described in basal cell carcinoma (Kerr & Searle, 1972), as well as a variety of normal mammalian tissues (Kerr, Wyllie & Currie, 1972). The rate at which apoptosis occurs will alter population size and could be an important point of action for a regulatory mechanism. 'Apoptotic cells' are not often seen in normal unstimulated epidermis, but focal cell death is not uncommon after epidermal injury from trauma or disease (eg. ultraviolet light, sticky tape stripping, erythema multiforme, pityriasis lichenoides). The curious focality of their death prompts the question—are these cells primed to die under stress? After all, the mechanism (apparently present in fish) will be part of the phylogenetic memory. If this is the case, apoptosis would be an important homeostatic mechanism designed to prevent wild swings in population size after unexpected stimulation.

Langerhans cells are dendritic cells found in the middle of the epidermis. Their function was totally obscure until comparatively recently and it was suggested that they may have a role in controlling proliferation in the 'epidermal proliferative unit' (Mackenzie, 1975). The later demonstration of an important immune function suggests that this probably is not the case.

The removal of stratum corneum by stripping causes a burst of mitotic activity which peaks at approximately 48 h in man (Griffiths & Marks, 1973). Is this due to release of a stimulatory substance, or the physical removal of the stratum corneum itself? The usual contacts of the skin with the environment—the rubs, scratches and knocks and the commonplace toilet procedures such as bathing—also remove stratum corneum, though in a somewhat less predictable manner than from tape stripping. If the assumption that epidermal volume is constant in health is correct, then presumably any sudden increased losses of corneocytes are compensated for by transient increases in mitotic activity. Work in progress suggests that completely protected but otherwise normal epidermis has approximately half the mitotic activity of comparable unprotected epidermal tissue. It is possible that the external environment itself plays an important role in determining the usually observed replicative activity of the epidermis and in this sense should be considered 'a regulator'.

Epidermal cells enlarge as they mature (Yardley & Goldstein, 1976; Marks & Barton, 1980). In steady states the volume changes that cells undergo during maturation are of no consequence to the equation of cell production equals rate of cell loss. However, if something perturbs cell volume interrelationships (e.g. by differentially affecting keratinization) profound changes may occur in rates of production and/or cell loss to maintain the overall epidermal volume.

CONCLUSION

Over the past two decades it has been suggested that some application of the 'negative feed back principle' controls epidermal population size and mitotic activity. The variety of physiological stimuli that can influence mitotic activity and the availability of other mechanisms provoke doubt as to the importance of any one control mechanism. Three fundamental deficiencies prevent better understanding of the regulation of epidermal population size: the lack of precise and convenient methodologies, the lack of data on normal human epidermal dynamics due to inadequate and uncritical experimentation, and the lack of detailed examination of current assumptions. The first is unpreventable, the second regrettable and the third unpardonable.

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British Journal of Dermatology (1980) **103,** 703.

Obituary

DR J.C.HAY

Dr Jannette Ceridwen Hay, formerly Consultant Dermatologist at the Royal Liverpool Children's Hospital, Whiston Hospital, Walton Hospital and Maelor General Hospital, died on 20th January, 1980, at the age of 69.

Netta Hay was born in Birkenhead of Welsh stock and was Welsh-speaking. She was educated at the County School, Bangor, then studied medicine at the University of Liverpool, where she qualified in 1934. She subsequently held appointments in the Liverpool Royal Infirmary and then showed her lifelong interest in Paediatrics by holding hospital posts in the Royal Liverpool Children's Hospital and the Princess Elizabeth of York Hospital, London.

She was appointed Holt Fellow in Physiology in the University of Liverpool and did research on the blue-green spectrum which formed the basis of her MD thesis awarded in 1938. During this period she still found time to be clinical assistant to Dr Norman Capon at the Royal Liverpool Children's Hospital, and she also became clinical assistant to Dr R. Stopford-Taylor in the Skin Department at the Liverpool Royal Infirmary. In 1939 she was appointed Honorary Physician at the Royal Liverpool Babies Hospital but on the outbreak of World War II she found herself working as locum consultant in both Paediatrics and Dermatology with sessions at the Royal Liverpool Children's Hospital, Birkenhead and Wirral Children's Hospital, the Royal Southern Hospital, Whiston Hospital and Wrexham Emergency Hospital. With the end of the war she had to choose between Paedriatrics and Dermatology and, fortunately for those in the latter specialty, that one was her choice, so with the inception of the NHS she became

Consultant Dermatologist at the hospitals where she had worked during the war, except for the Royal Southern Hospital; she was instead appointed to the staff of Walton Hospital.

In the early 1960s when the Dermatology Departments of the Liverpool teaching hospitals were centralized in the Liverpool Royal Infirmary, Netta added to her burden by establishing, on a voluntary basis, a contact dermatitis clinic which was continued until her successor was appointed in 1975. She carried on working at the Maelor General Hospital and Whiston Hospital almost to the end of her life relinquishing these commitments only when she became bedridden. An excellent diagnostician, she had an incredible memory for cases and people seen many years previously and her opinion was regularly sought over difficult clinical problems. She had to be asked, however, because although full of energy and life, Netta was extremely shy and modest and would never proffer an opinion uninvited. The same characteristics made her claim not to like teaching but she was nevertheless an excellent and popular instructor of both undergraduates and postgraduates and she took an active part in a comprehensive undergraduate teaching programme which she continued doing to the time of her final illness. Although she was a regular attender at the meetings of the North of England Dermatological Society and the British Association of Dermatologists her innate shyness allowed her to make only rare public utterances and perhaps she was not as well known outside Liverpool as she deserved to be.

Always elegant, vivacious and charming Netta also was generous and kind, especially to her juniors, and her enthusiasm and encouragement influenced several young men and women to take up Dermatology. Away from work Netta's great love was her family but she was also a keen and knowledgeable gardener; she travelled widely with her husband and with him walked the length and breadth of the Lake District. She greatly enjoyed entertaining in her beautiful home set on the northern bank of the River Dee with a marvellous and ever-changing view of the Welsh hills, a scene she found very restful in the later stages of her illness.

Together with her husband, son, two daughters and eleven grandchildren we grieve for her passing and those of us privileged to have known her will remember her with affection and respect.

T.W.STEWART

British Journal of Dermatology (1980) **103**, 705.

Correspondence

Desquamative gingivitis: a form of cicatricial pemphigoid?

SIR, We would like to comment on the article by Fine & Weathers (British Journal of Dermatology (1980) **102**, 393). The authors refer to an article by us, stating that the title we used—'Acquired oral non-dystrophic epidermolysis bullosa'—was a cumbersome one (British Journal of Dermatology (1977) **96**, 111).

We wish to restate that the term desquamative gingivitis (gingivosis), used by Fine & Weathers, should no longer be considered as a definitive diagnosis. McCarthy, McCarthy & Shklar (1960) have already pointed out that a wide range of diseases, including cicatricial mucous membrane pemphigoid, erosive lichen planus and pemphigus vulgaris, may masquerade under this outdated descriptive title.

Our suggested term may be cumbersome, but at least it contains the principal clinical characteristics of this group of patients. The condition appears to be an acquired one, confined to the mouth, with bullae and erosions being easily produced on surfaces exposed to friction or trauma, and healing occurring without scar formation. The traumatic factor appears to be an important one.

Fine & Weathers also refer to an intraoral Nikolsky's sign. We feel that they have misapplied the term Nikolsky's sign, which should be regarded as an expression of epithelial stripping or dissociation and which occurs in conditions such as pemphigus vulgaris and foliaceus and in toxic staphylococcal epidermal necrolysis, but not in subepithelial bullous lesions.

The continued search by many workers for auto-antibodies in the past 6 years has widened the field of positive findings in bullous disorders. Since our 1977 article, one of us (F.N.) has seen forty similar patients. Twenty-five were tested for antibodies in the basement membrane zone and twelve were positive. However, in epidermolysis bullosa, positive basement membrane auto-antibodies have been demonstrated (personal communication from Dr A.Marsden, St John's Hospital, London). Therefore it would be premature to assume that a positive basement membrane antibody in these cases is indicative of either bullous pemphigoid or cicatricial mucous membrane pemphigoid. It is also of interest that the basement membrane zone could not be demonstrated histologically with PAS stain in our series. We would be interested to know if Fine and Weathers were able to confirm this.

Finally, our experience with a relatively large group (now over fifty) over many years has convinced us that the condition is not cicatricial mucous membrane pemphigoid. It can undergo spontaneous remission, it is non-progressive, non-scarring and limited to the mouth (not necessarily to the gingiva). For the above reasons, we feel that the term 'acquired oral non-dystrophic epidermolysis bullosa' is still at present more suitable than 'desquamative gingivitis' for this intriguing condition.

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McCARTHY, F.P., McCARTHY, P.L. & SHKLAR, G. (1960) Chronic desquamative gingivitis: a reconsideration. *Oral Surgery*, **13**, 1300.

Reply

SIR: We have read with interest the letter from Doctors Forman and Nally and find very little of substance with which to disagree. We wholeheartedly agree that desquamative gingivitis may very well be a distinct entity and not a form of cicatricial pemphigoid even though the histology and immunofluorescence are similar. In fact, this is the central theme of our paper. We also agree with Doctors Forman and Nally that the positive basal membrane zone immunofluorescence alone does not conclusively establish the diagnosis of cicatricial mucous membrane pemphigoid, nor for that matter any other entity. We tried to make this point very clear.

The discussion of the intra-oral Nikolsky sign which they say we have misapplied depends on one's concept of what Nikolsky really described. It is true that Nikolsky originally referred to the intra-epithelial disassociation that occurred in pemphigus foliaceus with pressure on a bulla. The term has also been applied to dermal epidermal separation by pressure in conditions with decreased adhesiveness at this interface (Arndt & Feingold, 1970). Stripping the wall mucosa from basal lamina can be induced in desquamative gingivitis by finger pressure, manipulation during biopsy, or with an air syringe, a modified Nikolsky sign if you will.

It is true that in the strictest sense the lesions in desquamative gingivitis do not desquamate; however, the term is ingrained in the literature and the merit of changing term merely for the sake of change is questionable. The term, 'acquired oral non-dystrophic epidermolysis bullosa', is still in our opinion a cumbersome term, unnecessarily long and may or may not be an accurate assessment of the true nature of the disease. Use of the term, 'acquired epidermolysis bullosa' really does not clarify matters since the pathogenesis of this disorder has not been completely elucidated. Since a rose by any other name would still smell as sweet, we see no real reason to substitute a long new term for a short old one which is recognized by the great majority of clinicians.

Our findings with regard to PAS stains in 'desquamative gingivitis' are variable. In our experience the basement membrane zone does not always stain distinctly. At times, positive staining is present in the roof of the bulla and/or on the connective tissue surface. This may indicate a different pathogenetic mechanism or may merely be a quantitative difference in the degree of inflammation and subsequent damage.

In summary, we agree that desquamative gingivitis is a clinical term most appropriately used to describe gingival involvement with a vesiculo-erosive disease which peels with very minor manipulation and has other distinctive clinical features. In spite of the similar histological and immunofluorescent findings, it is probably not a form of cicatricial pemphigoid but rather it shares features with other mechanicobullous disorders. Its exact aetiology and pathogenesis are currently unknown and must be clarified by further study. We are currently investigating the mechanism of dermal epidermal separation using immunoelectronmicroscopy. Until the cause of this disorder is clarified it seems futile to argue about its nosology.

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Psoriasis and the virus

SIR, Until quite recently, family clustering over a number of generations has been accepted as evidence that a disease is the result of a Mendelian dominant gene. It is now recognized that this may not always be true, and that several disorders previously regarded as 'genetic' are in fact caused by the vertical transmission of a virus in early childhood (Harper, 1977; Ford, 1978). Further, the viral DNA may remain dormant in the cell for long periods, translation to viral protein not occurring until much later in life. Thus electron microscopy and even culture techniques may fail to provide any indication of the original infection.

The implications for psoriasis are alarming. Are we never to be rid of the spectre of a viral aetiology? Fortunately, as the above authors point out, one valuable test remains: if early exposure to an infectious agent is a necessary event in the pathogenesis of a disease, then maternal transmission will predominate over paternal transmission. We have therefore analysed the data of Lomholt (1963) for evidence of such an anomaly. His family pedigrees yield 451 children born to parents where the mother only had psoriasis; of these, 106 were affected. Similarly, of 428 children born to parents where the father only had psoriasis, 114 were affected. The difference is not significant (χ^2-test). It would seem that psoriasis is, after all, not a virus disease.

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British Journal of Dermatology (1980) **103**, 708.

Book Reviews

Photochemical and Photobiological Reviews. Volume 5. Edited by KENDRIC C.SMITH (1980). New York: Plenum Publishing Corporation. Pp. 316. Price $35.00.

This 5th volume in a regular series of photobiologically oriented reviews, well presented by Plenum Press and their editor, Kendric Smith of Stanford University Medical School's Department of Radiology, follows the excellent tradition established in 1964 by Arthur Giese and Academic Press with their Photophysiology series. It is, therefore, particularly pleasing to see in this volume a delightful review by Giese on one of his pet subjects, Hypericism, the photosensitivity skin disease of domestic animals induced by hypericin, a red pigment contained in weeds of the genus *Hypericum*. Although of little direct interest to Dermatology, this is a good treatment of the nature and mode of action of an exogenous photosensitizer, and British Dermatologists may have some added affection for the subject matter because the prime culprit involved is *Hypericum perforatum*, the Common St John's Wort. Of more direct and topical concern in this volume is Margaret Kripke's review of the immunological aspects of ultraviolet carcinogenesis. This briefly covers tumour antigens generally and those involved in photocarcinogenesis in particular and also the effects of ultraviolet radiation on various immunological functions such as DNCB reactivity and graft rejection. However, the review most properly centres on the role of the immune system in ultraviolet carcinogenesis as effected through the induction of suppressor T lymphocytes which specifically inhibit the rejection of UV-tumour cells. This work, much of it done by Dr Kripke and her colleagues, is the most exciting in the field since the finding of reduced repair capability in the cells of patients with xeroderma pigmentosum.

Three other chapters may interest those working on the molecular and cellular mechanisms involved in ultraviolet radiation effects. These are an extensive review of UV-induced DNA-protein cross linking, a photochemical lesion which remains overshadowed by the more popular pyrimidine dimer but may have important biological consequences; a concise discussion of the immunological detection of radiation damage to DNA; and an excellent treatment of the use of fluorescent probes in the study of nucleic acids and chromatin in living cells.

The remaining chapters on photosynthesis, circadian rhythms and Photobiochemistry in the dark (sic) are well done but of rather specialist interest. Any department which specializes in photobiology will certainly purchase this book but, even with the Kripke review, it cannot be recommended for the general dermatological collection.

B.E.JOHNSON

Research Monographs in Immunology, Vol. I. Delayed Hypersensitivity. J.L.TURK (1980). Amsterdam & New York: Biomedical Press. Pp. xii+308. Price $65.75.

This book is in fact a third edition and was previously published by Elsevier under the title North Holland Research Monographs of Biology No. 4 in 1975. The significant additions to the 1975 edition are the inclusion of an extra chapter on the role of delayed hypersensitivity in granuloma formation and the inclusion of a reference—regrettably only one—to the Langerhans cell.

The text remains an important source book for those with an interest in the laboratory rather than the clinical aspects of delayed hypersensitivity, but investigationally-inclined clinicians would also benefit from careful study of the text prior to initiating clinical studies. The section on skin testing to DNCB and DNFB in Chapter 2 should be required reading in such cases.

Other topics covered in a scholarly and thoughtful manner include the homograft reaction, tolerance and autoimmunity. The new chapter on granulomata is a clear and enjoyable account of this common dermatological problem. Perhaps the next edition will include a similar addition on recent work on the Langerhans cell.

RONA M.MACKIE

Allergy: Principles and Practice. Edited by E.MIDDLETON, JR., C.E.REED AND E.F.ELLIS (1978). St Louis: C.V.Mosby. Pp. 1182. Price £61.00.

This very handsome two volume textbook of allergy covers all the conventional aspects of allergy without attempting to include the whole field of clinical immunology—and very well it does it.

There are fifty-two contributors, mainly from the United States, and the editors rightly claim to have secured 'a truly outstanding "star-studded" cast'.

Unlike many textbooks of clinical allergy there is here a much greater emphasis on basic mechanisms. Indeed the whole of the first volume describes not only the relevant basic immunology but also the basic physiology and pharmacology. Inevitably much of the emphasis throughout the two volumes is on respiratory allergies but diseases of the skin, eye and gut, anaphylactic and drug reactions all receive good mention.

Many chapters do not just give a didactic account but provide historical perspective and give a full critical review of the literature, for example on specific hyposensitization with 116 references.

There is much in this book of relevance to dermatologists. The basic mechanisms, epidemiology and genetics of atopy for example are dealt with more fully than in dermatology texts, the more obviously clinical chapters in rather less detail but with a slightly different slant. There is much in this book too to confirm that allergy both scientific and clinical can be fully respectable. Not everything is claimed for allergy, which by definition here must be able to demonstrate an immunological mechanism and characterize it.

R.H.CHAMPION

ERRATUM

Diagnosis of Skin Disease. GERALD S.LAZARUS and LOWELL A.GOLDSMITH with the assistance of MICHAEL D.THARP (1979). Philadelphia: F.A.Davis.

In *British Journal of Dermatology*, **103**, 2, the price for this book was quoted as £3.25. The correct price is £37.50.

British Journal of Dermatology (1980) **103**, 710.

Annual Meeting of the British Association of Dermatologists

The 60th Annual Meeting of the British Association of Dermatologists was held in Oxford from 9 to 12 July 1980 under the Presidency of Dr Darrell Wilkinson; the local Secretary was Dr R.P.R. Dawber. More than 450 members and guests were in attendance. We were pleased to welcome as official guests this year the International League of Dermatologists, many of whom were accompanied by their wives.

The British Association of Dermatologists was pleased to be associated with the inaugural meeting on 8 July of the British Contact Dermatitis Group, under its Chairman, Dr Richard Rycroft, and secretary, Dr John Wilkinson. The British Society for Dermatopathology (President, Dr Martin Black) held a very successful one-day meeting, organized particularly with trainee dermatologists in mind, on Wednesday 9 July.

The Annual General Meeting of the B.A.D. was held on Wednesday afternoon, 9 July.

The scientific presentations were given at the Academic Centre, John Radcliffe Hospital. Twenty-six papers were presented plus three guest lectures by Professor Neils Hjorth, Professor C.E.Orfanos and Professor R.W.Winkelmann.

The official reception was held at Pembroke College on Thursday 5 July.

The social events included a one-day visit to Woburn Abbey on Thursday 10 July, an afternoon visit to Waddesden Manor and a boat trip on the Thames on Friday 11 July. The annual dinner was held at Keble College on 11 July when 330 members and guests were in attendance.

The Dowling-McCaw Golf Cup was won by Dr Dai Roberts and Dr Wilson Taylor. The Bowers/Sneddon Sailing Trophy was won by Drs Peter Goodwin and Bill Bowers. An innovation this year was the daily early morning 2½ mile run in The Parks; the prize for the fittest and most proficient runner was won by Dr David Dick.

There were twenty-six entries in the scientific poster exhibition and the Bristol Cup was awarded to Drs Tony Chu and Donald MacDonald of Guy's Hospital.

The Clinical Meeting was held at the John Radcliffe Hospital on Saturday 12 July; forty-two cases were presented and discussed.

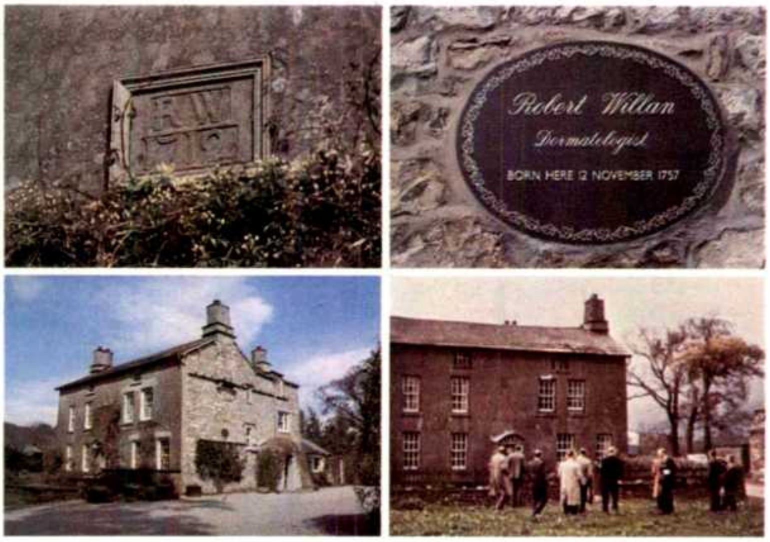
Robert Willan Plaque

Readers of this Journal will be familiar with the picture of Robert Willan on the cover, designed by Dr Francis Ray Bettley from a portrait. Through the good offices of the late Professor John T.Ingram, this portrait, bequeathed to Sedbergh School, is now on permanent loan outside the Willan Room in the Royal College of Physicians, London.

Dr Bettley has now produced another excellent piece of work, in the form of a plaque, which has been erected on a wall of Willan's birthplace. 'The founder of British dermatology' was born in a farmhouse called 'The Hill' near Sedbergh, Yorkshire. His father, also Robert Willan, was a well-known physician who graduated in Edinburgh in 1745, and he himself, 'A first-rate classical scholar', graduated M.D.Edin. in 1780, and was admitted as a Member of the Royal College of Physicians, London, in 1785.

The investigations of E.K.Morris, F.R.C.P.Ed., physician to Sedbergh School where Willan was educated, revealed that our subject was born at The Hill. When sixteen of our members visited the house on the 12th May, 1962, we noticed on the house a stone bearing the initials 'R.W.' and the date 1712, while on the large barn was the same 'R.W.' with the date 1748. For over 300 years The Hill belonged to the Willan family, but fell into sad disrepair until bought by Mr L.P.Madge, M.A., Senior Biology Master at Sedbergh School; he and his wife have done a great deal towards the preservation of this charming old house.

In a letter dated 26 June, 1971, he said '... my wife and I would certainly be very willing that a suitable plaque be placed on the house, and, as I have already indicated, am most anxious that members of your Association and other duly qualified dermatologists shall have free access to the house, provided, of course, in so far as it is possible, we were warned of pending visits ... we would do our very best to ensure that if we sold the property the future owner would be asked to continue the above access arrangements'.



The plaque has recently been set up; and photographed by Dr R.H.Seville. An unveiling ceremony was held on 25 October 1980. The colour plate shows a close-up of the 1712 plaque, a close-up of the new plaque, a general view of the house with the new plaque installed, and also a photograph taken in 1962 when the Willan Club visited The Hill (left to right Drs Hellier, Mitchell-Heggs, Frain-Bell, Garretts, Freshwater, Bettley, Peterkin, Anderson, Fergusson).

G.A.GRANT PETERKIN

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News and Notices

University of Colorado Sixth Annual Winter Skin Seminar

This seminar, held at The Given Institute of Pathobiology, Aspen, Colorado, February 8–13 1981, is designed to provide the practising dermatologist with a review and update of dermatological therapeutics, and to present a balance of clinical and scientific presentations relevant to the practice of dermatology. Projected slides and handout material will be utilized. The goal of the seminar is to provide both practical information which can be used in the daily practice of dermatology and more basic information which will aid the participant in passing the self-assessment examination of the American Academy of Dermatology or the American Board of Dermatology examination.

For information or registration, contact the Office of Postgraduate Medical Education, The University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, Colorado, 80262.

International Society of Tropical Dermatology

The next meeting of the International Society of Tropical Dermatology will be held in Cologne, April 1–4 1981, in The Lecture Building (Hörsaalgebäude) of the University, Albertus-Magnus-Platz, Köln 41.

The general theme will be new developments in dermatology with special focus on tropical and subtropical countries.

The main subjects will include:

(1) Therapy (retinoids, corticosteroids, acne therapy, leprosy).
(2) Mycoses (candidosis, systemic mycoses, actinomycoses).
(3) Photodermatoses and phototherapy.
(4) Virology (new therapeutic aspects, ticks as transmitters, herpes, warts).
(5) Oncology (melanoma, lymphoma).
(6) Contributions to current problems, and free communications.

Chairman of the meeting is: Prof. Dr G.K.Steigleder, Direktor der Universitäts-Hautklinik Köln, Joseph-Stelzmann-Strasse 9, D-5000 Köln 41, Germany.

Correspondence and submissions of short communications (10 minutes) should be sent to Professor Steigleder before the 1st January 1981.

The Cologne meeting will be followed by a meeting—9–11 April in Copenhagen.

Paediatric Dermatology Course

A course in Paediatric Dermatology will be held at Arcachon, near Bordeaux, France, 21st–25th April 1981, organized by Professor M.Larregue and Professor J.Maleville.

Subjects to be discussed include: hypomelanosis, hypermelanosis, poikilodermia, pathology of polymorphonuclear leucocyte, and Skin and aminoacidopathy.

There will be a clinical meeting in Bordeaux.

Enquiries may be made to: Secretariat du Service de Dermatologie, Hopital des Enfants, 168 cours de l'Argonne, 33077 Bordeaux-Cedex.

Annual Meeting of ESDR 24–27 May 1981

The 1981 Annual Meeting of the European Society for Dermatological Research (ESDR) will be held in the Leuwenhorht Congress Centre, Noordwijk, Holland, 24–27 May 1981.

Attendance at scientific sessions will be open to all members, contributors and guests. Non-members who wish to present a paper may submit an abstract. They will be invited to attend the meeting as either contributors or guests.

Abstracts should be presented on the official form. The deadline for submission of abstracts is 15 January, 1981. Abstract forms can be obtained from: Professor M.W.Greaves, Secretary—ESDR, Institute of Dermatology, Homerton Grove, London E9 6BX.

8th Annual Meeting of the Society for Cutaneous Ultrastructure Research (SCUR)

The 8th Annual Meeting of SCUR will be held at Guy's Hospital, London, England, from 21 to 23 May, 1981.

Dermatologists, Pathologists and other interested scientific workers are invited to participate. For details and registration forms please write to: Dr D.M.MacDonald, Secretary of the Organizing Committee, Department of Dermatology, Guy's Hospital, St Thomas' Street, London SE1 9RT.

British Association of Dermatologists Summer Meeting 1–4 July 1981

Members are requested to provide cases for the Clinical Meeting on 4th July. Case histories should be written in flowing English with verbs. Investigations should include units and other matter, as set out in Notes to Contributors in the British Journal of Dermatology.

Please send two copies of the manuscript, which should be typed in double spacing with a broad margin, to Dr Michael Klaber at the Department of Dermatology, The London Hospital, Whitechapel, before 1 March 1981.

APPOINTMENTS

The Royal College of Physicians of Edinburgh: Royal College of Physicians and Surgeons of Glasgow

The following dermatologists have recently been elected to the Fellowship of the Royal College of Physicians of Edinburgh: Dr J.N.Burry, Dr A.G.Harper, Dr B.K.H.Nair and Dr H.Woolfson.

Dr M.K.Attia has been elected to the Fellowship of the Royal College of Physicians and Surgeons of Glasgow.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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